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CASE REPORT

CRIMINALISTICS

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The Conviction of Dr. Crippen: New Forensic Findings in a Century-Old Murder

ABSTRACT: Dr. Hawley Crippen was accused and convicted of murdering his wife in London in 1910. Key to the conviction was microscopic analysis of remains found in the Crippen's coal cellar, which were identified as Cora Crippen based on a scar she was said to have. Dr. Crippen was hanged, always proclaiming his innocence. In this study, genealogical research was used to locate maternal relatives of Cora Crippen, and their mitochondrial haplotypes were determined. Next, one of the pathology slides of the scar was obtained, DNA was isolated, and the haplotype was determined. That process was then repeated. Finally, both DNA isolates were assayed for repetitive elements on autosomes and repetitive elements specific to the Y chromosome. Based on the genealogical and mitochondrial DNA research, the tissue on the pathology slide used to convict Dr. Crippen was not that of Cora Crippen. Moreover, that tissue was male in origin.

KEYWORDS: forensic science, genealogy, mitochondrial DNA, sex determination, ancient DNA

Hawley Harvey Crippen was born in Coldwater, Michigan, in 1862 and went on to be at the center of one of the most infamous murders in British history. Although the details of the case have been told in more than 40 books and films over the years, including many detailed works (e.g., [1–14], from which the historical information below was obtained), serious questions remain as to his guilt or innocence. Crippen obtained a degree in homeopathic medicine in 1882, followed by additional education to qualify as a homeopathic physician in the same year. His first wife, Charlotte Jane (*nee* Bell) Crippen, died from "apoplexy," and their only child, Otto Crippen, was sent to live with his paternal grandparents. In 1892, Dr. Crippen married his second wife, Cora Mersinger. Cora Crippen was originally Kunegunde Mackamotzki, her surname changing to Mersinger upon her father's death and mother's remarriage. On her marriage certificate, she used the name Cora (Corinne) Turner and later took the name Belle Elmore for her stage career. She was 11 years younger than Dr. Crippen.

The Crippens moved to London in 1900 where Dr. Crippen represented the Munyon Homeopathic Home Remedy Co. On September 21, 1905, they moved into a rental property at 39 Hilldrop Crescent, Holloway, North London. Cora Crippen was last seen alive by friends visiting the Crippen home, at approximately 1:30 AM, on February 1, 1910. After her disappearance, Dr. Crippen was regularly accompanied by his office secretary, Ethel LeNeve, with whom, and to his wife's knowledge, he had been carrying on an affair for the past 3 years. Miss LeNeve was even seen wearing Cora Crippen's clothes and jewelry in public. When Mrs. Crippen's friends became suspicious over her absence, they took their concerns to New Scotland Yard and requested an investigation. The case was placed under the auspices of Chief Inspector Walter Dew, who interrogated Dr. Crippen about the location of his wife. Dr. Crippen stated that after their friends left on the evening she was last seen, he and his wife had an argument during which she said she was leaving him and was never seen again. It was Dr. Crippen's belief that she had run off with another man, one Bruce Miller, with whom she was thought to have had a romantic relationship.

On July 9, 1910, 1 day after being interrogated by Inspector Dew, Dr. Crippen traveled from London to Belgium accompanied by Ethel LeNeve, who was disguised as a boy; a few days later, they booked passage on a steamship and sailed for Canada. Inspector Dew returned to the Crippen's home on July 11th and found the residence empty. An extensive investigation into the property was carried out by New Scotland Yard detectives seeking any evidence that might shed light on the missing woman. Four days later, after removing a few floor bricks in the coal cellar and digging down several inches, decaying tissues were discovered, along with some female clothing, a man's pajama top, and bleached blond hair in curlers. The remains consisted of flesh with adipocere and viscera (heart, lungs, trachea, esophagus, liver, kidneys, spleen, stomach, pancreas, small intestines, and most of the large intestine). All of the organs had been removed en masse. No head, limbs, bones, or reproductive organs were found. The conclusion of the detectives was that these were Cora Crippen's remains, and a worldwide alert was issued to find Dr. Crippen and his mistress.

A toxicological analysis of the remains by a New Scotland Yard analytical chemist identified the alkaloidal substance hyoscine hydrobromide (scopolamine) in an amount determined to be lethal.

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The same drug had earlier been procured by Dr. Crippen at his local chemist for use in his homeopathic preparations. It was thus assumed that he had murdered his wife using this chemical, mutilated her body, disposed of the bulk of the remains in an unknown manner, and buried the rest in the cellar of his home.

A Marconi wireless message from Captain Henry Kendall of the S.S. Montrose was received by New Scotland Yard on July 22nd, stating that he was certain the sought couple were passengers on his ship. Inspector Dew booked passage on the faster S.S. Laurentic and arrived at the mouth of the St. Lawrence River a day ahead of the S.S. Montrose. Crippen and LeNeve were arrested the next day. The gruesomeness of the apparent murder, and subsequent chase across the Atlantic, led to Dr. Crippen's arrest being labeled the paramount one in British policing history (15).

Dr. Crippen and Ethel LeNeve returned to London to stand trial for murder. Imperative to the prosecution was identifying the cellar remains as those of Cora Crippen. Pathologist Bernard Spilsbury testified that based on histological analysis, a mark found on the recovered tissue was consistent with an abdominal surgery scar that Cora was said to have had. In addition, the label on the pajama top was dated later than 1905—after the Crippens had moved into the Hilldrop home. Dr. Crippen's trial lasted 5 days, and on October 22nd, after 27 min of jury deliberation, he was found guilty of the murder of his wife and sentenced to hang. LeNeve was acquitted in a separate trial.

Dr. Crippen continually protested his innocence although his appeals for a mistrial and clemency were denied. He was hanged at Pentonville Prison on November 23, 1910, and his body was buried on the prison grounds. Today, forensic methods exist that have the potential to establish whether the coal cellar remains were consistent with those of Cora Crippen, conditional upon two things: locating and genetically analyzing any portion of the remains still in existence and finding a known sample, either from Cora or from an extant relative, for genetic comparison. The research presented here provides the scientific details of those undertakings.

Materials and Methods

Identifying Maternally Related Relatives of Cora Crippen

Cora Crippen was unable to bear children; thus, no direct descendants of hers exist today. Owing to this, maternal relatives were sought who would allow for mitochondrial DNA (mtDNA) comparisons with the buried remains. A wide variety of resources were examined and utilized during the genealogical search for maternal relatives. These included the following:

- Soundex indices of the family name, which gave various spellings of Mersinger.
- Baptismal/christening records (birth records were not yet mandated, but sometimes existed).
- 1870 federal census records for Cora's family.
- 1880, 1890, and 1900 federal census records for Cora and family (the 1890 records were mostly destroyed by fire).
- Marriage records of Cora and any siblings.
- Death records of Cora's parents and siblings.
- Newspaper obituaries of any Mersingers or alternative spellings.
- Social security death records.
- Probate records.
- Transcripts from the murder trial (15).
- Living family members' records, recollections, mementos.
- Genealogical websites.
- Conversations with living relatives.

Obtaining Tissues from the Coal Cellar Remains

Tissue samples from the coal cellar exist in two places: New Scotland Yard's Police Evidence Museum, which possesses the hair found in curlers in the grave, and the Royal London Hospital Archives and Museum, which houses Dr. Spilsbury's slides used to identify the scar on the torso and thus to identify the remains as Cora Crippen. Owing to concerns about transport via commercial carriers and chain of custody, a hair was not offered for testing. In contrast, the Royal London Hospital Archives Committee agreed to send one of Dr. Spilsbury's nine available pathology slides (Fig. 1) to Michigan State University for DNA testing, realizing that such testing is destructive in nature.

DNA Isolation

All DNA processing was conducted using full personal protective equipment, and supplies were autoclaved, filter-sterilized, and UV-irradiated (at least 5 J/cm^2) as appropriate. Buccal swabs from three maternal relatives of Cora Crippen were received, DNA was isolated, and mtDNA sequences were generated 17 months prior to the time the Spilsbury slide was received and processed. One-half

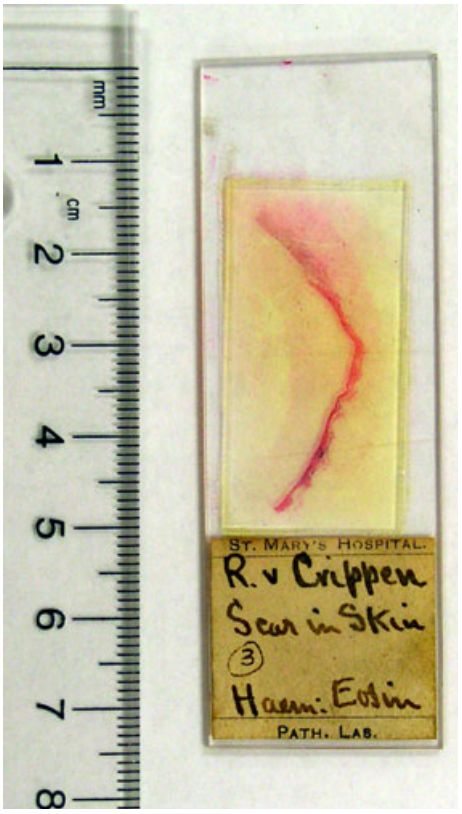


FIG. 1—*The tissue section from which DNA was isolated. The tissue, taken from the Crippen cellar remains, was used to identify them as originating from Cora Crippen. This slide was sent to Michigan State University from the Royal London Hospital Archives and Museum, where the slides produced by Dr. Spilsbury are housed. The case (R. v Crippen), histological stains used (hematoxylin and eosin), slide number 3, and Dr. Spilsbury's conclusion (scar in skin) are clearly noted. The presumed scar was stained red in color, while the cover slip was held on with a yellowish adhesive, likely pine resin. Three more slides also labeled "Scar in Skin," three of "Fold in Skin from Abdomen," and two labeled "Margin of Skin Abdomen" are also found at the Royal London Hospital Archives and Museum.*

of each buccal swab was cut and placed in 200 μL of digestion buffer (10 mM Tris pH 7.5, 20 mM EDTA, 0.1% SDS), along with 2 μL of 20 mg/mL proteinase K. Swabs and a reagent blank were incubated overnight at 55°C. Swabs were transferred to a spin basket, and liquid was removed via centrifugation, which was combined back with the remaining digestion buffer. An equal volume of phenol was added, and the tube was vortexed and centrifuged for 5 min at 20,000 × *g*. The aqueous layer was transferred to a new tube, an equal volume of chloroform was added, and the procedure was repeated. DNAs were precipitated by the addition of 0.1 vol of 3 M sodium acetate and 2 vol 95% ethanol. Samples were stored at −20°C for 1.5 h and then centrifuged at 20,000 × *g* for 15 min. Liquid was removed, and the DNA pellet was vacuum-dried. Pellets were resuspended in 30 μL 10 mM Tris pH 7.5, 1 mM EDTA (TE).

DNA from the fixed tissue was isolated based on several publications (e.g., [16,17]), with the methodology detailed in Chamberlain (18). The slide was immersed in xylene in an attempt to loosen the cover slip. As this did not release it, small fragments of the cover slip were removed by chipping them away using a sterile scalpel, exposing the tissue. The formaldehyde-fixed tissue was scraped into a sterile 1.5-mL microfuge tube, to which 1 mL of xylene was added (19,20). A reagent blank was initiated. The tissue was soaked for 30 min, followed by centrifugation (20,000 × *g*) for 3 min. The xylene was removed and the procedure was repeated. The tissue was then soaked in 1 mL of 95% ethanol for 5 min and centrifuged, the ethanol was removed, and the procedure was repeated. The tissue pellet was dried under vacuum. Two hundred microliters of digestion buffer was added to the tube, along with 10 μL of proteinase K. The tube was vortexed and incubated at 55°C overnight. The liquid was transferred to a Microcon YM 30 (Millipore, Billerica, MA) and centrifuged at 10,000 × *g* for 10 min. The retentate was washed three times with 400 μL TE, and the DNA was recovered in 200 μL TE.

DNA was purified via both Chelex and organic extraction, given previous research that showed differential polymerase chain reaction (PCR)–based results between the two when performed on fixed tissues (18,19). The 200 μL of DNA was divided equally into two microcentrifuge tubes, the first of which was brought back to 200 μL using TE, followed by the addition of an equal volume of phenol. The tube was vortexed and centrifuged for 5 min at 20,000 × *g,* the aqueous layer was transferred to a new tube, an equal volume of chloroform was added, and centrifugation and transfer were repeated. The aqueous portion was purified on a Microcon YM 30 as detailed earlier, and DNA was recovered in 20 μL TE. Ten microliters of 5% Chelex (Bio-Rad, Hercules, CA) (16,19,21) was added to the remaining 100 μL of DNA, which was incubated at 55°C for 30 min, and then placed in boiling water for 8 min.

Following the original round of DNA analysis, more of the slide's cover slip was removed, and DNA extraction (using Chelex), DNA amplification, and DNA analysis were repeated.

DNA Amplification and Sequencing

DNAs from the buccal swabs were amplified using mtDNA primers F15989 and R569 (22). Reactions included 2 μM forward and reverse primer, 200 μM dNTPs, 2.5 mM $MgCl_2$ 1 unit DNA polymerase (Eppendorf, Westbury, NY), and 1X supplied buffer. One microliter of neat or 1:20 diluted DNA was added. PCR parameters were 94°C for 5 min, followed by 35 cycles of denature at 94°C for 30 sec, anneal primers at 55°C for 1 min, and extend at 72°C for 1.5 min. Five microliters of PCR product was separated on a 3% agarose gel, and concentration was estimated for sequencing.

mtDNA sequencing of the buccal DNAs was conducted using a CEQ DTCS Quick Start kit (BeckmanCoulter, Fullerton, CA) in 10 μL volumes using primers F15989, R16410, F15, and R569 (primer sequences except those noted can be found in Edson et al. [22]) following the manufacturer's protocol. DNAs were separated on a CEQ8000 (BeckmanCoulter), and resulting sequences were compared to the reference sequence (23) and to each other.

Primers for slide DNA amplification were chosen based on regions that showed uncommon polymorphisms in the buccal swab DNAs. Amplification parameters followed those above, except in some instances the annealing temperature was lowered to 50°C or raised to 57°C to augment primer binding or increase binding specificity, respectively. In some instances, 38 PCR cycles were performed. Amplification primer pairs included F16190/R16410, F15/R285, F15989/R16207 (the reverse sequence of F16190), F82 (5′–ATAGCATTGCGAGACGCTGG–3′)/R285, F16450/R16, and F256 (5′–CACAGCCACTTTCCACACAG–3′)/R484. One microliter of neat and 1:20 diluted DNA was amplified. For all mtDNA processing, negative, positive, and reagent blank controls were included.

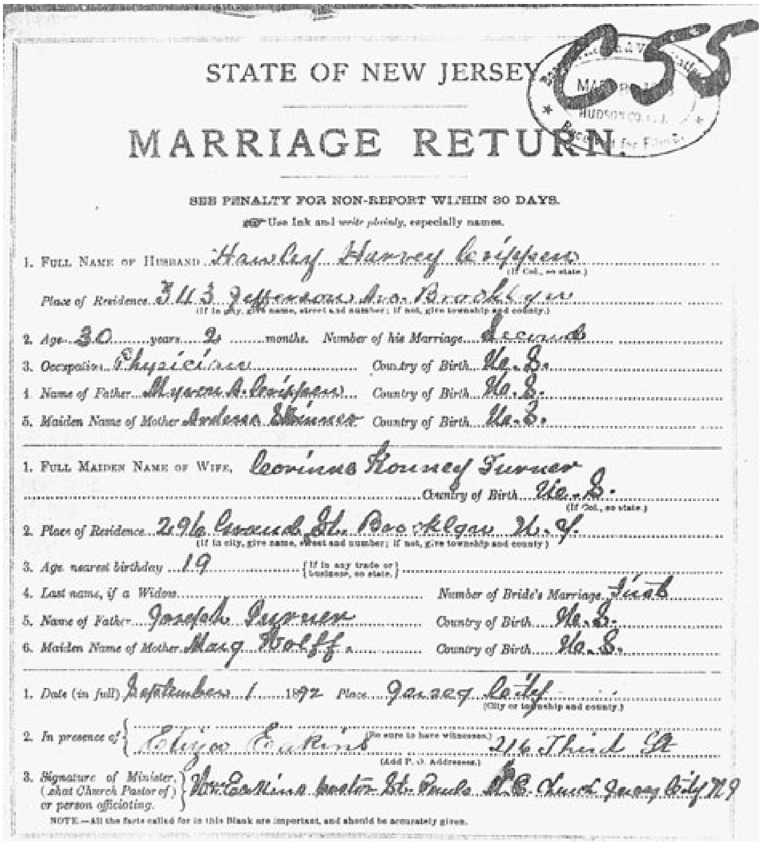
Sex Determination

Attempts to amplify amelogenin were negative (data not shown); therefore, sex determination of the slide tissue was accomplished through multiplex amplification of Y chromosomal and autosomal high copy number sequences (24,25). For the former, a 143-bp DYZ1 repeat sequence on the Y chromosome containing 2000–4000 copies (26) was amplified using the primers 5′–GGCCTGTCCA TTACACTACATTCC–3′ and 5′–GAATTGAATGGAATGGGAA CGA–3′, and the TaqMan probe 5′–6FAM-ATTCCAATCCATTC CTTT-MGBNFQ–3′ (24). A 127-bp repetitive sequence of the Ya5 Alu subfamily with similar copy number was amplified in tandem as a human/female DNA control, using the primers 5′–GAGATCGAG ACCATCCCGGCTAAA–3′, 5′–CTCAGCCTCCCAAGTAGCTG–3′, and the TaqMan probe 5′–DHEX-GGGCGTAGTGGCGGG-DBH1–3′ (27). Reactions, run in triplicate, contained 1x iQ5 Supermix (Bio-Rad), 500 nM DYZ1 forward, reverse, and Alu forward primer, 900 nM Alu reverse primer, 250 nM DYZ1 and Alu probes, 10-μg nonacetylated bovine serum albumin (Fisher Scientific, Pittsburgh, PA), 1 μL DNA or a 1:15 dilution of the DNA, and sterile water. Cycling parameters were performed on an iQ™5 Multicolor Real-Time Detection System and iCycler (Bio-Rad) and consisted of a 95°C hold for 10 min, and 50 cycles of a 95°C denaturing step for 15 sec and a 60°C annealing/extension step for 1 min (see [24]). Results were analyzed using the iQ™5 software. This procedure was then repeated on the second slide DNA isolation/purification outlined earlier. Negative, positive, and reagent blank controls were included; however, male control DNA was never assayed at the same time as DNA from the slide.

Results

Genealogical Findings

Cora Crippen (Kunegunde Mackamotzki) was born in Brooklyn, New York, in 1873, the daughter of Joseph Mackamotzki and Mary (Maria) Wolff. The date of Cora's birth was calculated from her and Dr. Crippen's marriage license in July of 1892 (age 19; Fig. 2) and is corroborated by census data (below). Her father died, and her mother married Frederick Mersinger by 1880.


STATE OF NEW JERSEY

MARRIAGE RETURN.

SEE PENALTY FOR NON-REPORT WITHIN 30 DAYS.

Use Ink and write plainly, especially names.

1. Full Name of Husband Hawley Harvey Crippen (If Col., so state.)

Place of Residence 343 Jefferson Ave Brooklyn (If in city, give name, street and number; if not, give township and county.)

2. Age 30 years 2 months. Number of his Marriage Second

3. Occupation Physician Country of Birth U. S.

4. Name of Father Myron A. Crippen Country of Birth U. S.

5. Maiden Name of Mother Andresa Skinner Country of Birth U. S.

1. Full Maiden Name of Wife, Corinne Turner Country of Birth U. S. (If Col., so state.)

2. Place of Residence 296 Grand St. Brooklyn N. Y. (If in city, give name, street and number; if not, give township and county.)

3. Age nearest birthday 19 (If in any trade or business, so state.)

4. Last name, if a Widow Number of Bride's Marriage First

5. Name of Father Joseph Turner Country of Birth U. S.

6. Maiden Name of Mother Mary Wolff Country of Birth U. S.

1. Date (in full) September 1 1892 Place Jersey City (City or township and county.)

2. In presence of (Be sure to have witnesses.) 216 Third St (Add P. O. Addresses.)

3. Signature of Minister, (what Church Pastor of) or person officiating. Pastor St. Pauls M. E. Church Jersey City N J

NOTE.—All the facts called for in this Blank are important, and should be accurately given.

FIG. 2—*The marriage license of Hawley and Cora Crippen. The couple was married in New Jersey in 1892, she at age 19, under the name Cora (Corinne) Turner, and he age 30, noting his occupation as a physician and that it was his second marriage.*

Mackamotzki/Mersinger children in the 1880 census records (Fig. 3*a*) included Cora ("Concardia"), age 7, along with A, age 13, T, age 4, and K, age 2. In the 1900 census records (Fig. 3*b*), family members grew to include F (born 1882), L (1885), Ju (1888), B (1893), and Jo (1895). Four of these (T, K L, and B) were girls and could be used to follow the maternal line. One (T) lived on Long Island and testified at the Crippen trial. Records indicate she had a sole son. K was married but also had only sons. L had one child of unknown sex. Finally, B bore a daughter in 1912 (Fig. 4) who died in 1973. Her daughter had four children, two of whom were located. Both provided buccal samples for testing, as did one of their daughters. These were the grandnieces and great-grandniece of Cora Crippen. The nieces had some knowledge of their aunt's murder, although it was reportedly unwelcomed conversation during their upbringing.

Several other living descendants of Mary Mersinger were located, although none came through a solely maternal lineage. These included a great-granddaughter of L, who had some awareness of the case, and a granddaughter of K who knew her grandparents' histories in the Mersinger family. Of greater interest was a granddaughter of Jo, who often spoke with her cousin, a daughter of F, about Cora's murder, and possesses a letter to Mary written from her Russian homeland. A son of F was also located, who vividly remembered the details of the murder and even possessed pictures of Cora in her stage costume.

Genetic Findings

The three maternal relatives of Cora Crippen produced identical mtDNA haplotypes, each differing from the reference sequence at seven positions (including the common 263G and 315.1C). The haplotype was unique in the FBI database (28). The polymorphisms allowed for more focused examination of mtDNA from the coal cellar remains, as smaller segments of the relatively degraded slide DNA could be preferentially examined to determine whether they shared them.

In no instances, did slide DNA purified via organic extraction amplify, in spite of repeated attempts. Given these results and those obtained previously (18), organic extraction was not attempted for the second DNA isolation from the slide. In contrast, weak but visible amplicons were produced from the original Chelex extraction. Attempts to intensify the product through lowering of the primer annealing temperature (see Methods) were generally successful; however, in some instances, the reagent blank also produced a (very weak) product; thus, these results were disregarded. Increasing the primer annealing temperature and cycle number resulted in clean amplification products, with no product from the reagent blank. Sequences were obtained across six of the seven polymorphic sites found in the grandnieces' mtDNA. Of these, the only shared polymorphisms were 263G and 315.1C; the sequence obtained from the slide matched the reference sequence at the other four sites and also contained 309.1C, and hence, differed at a minimum of five sites. These results were consistent in the second round of DNA isolation and amplification, with no amplification observed in the reagent blanks.

The real-time sexing assay produced clear results from the slide and control DNAs. Female control DNA generated a positive signal for the autosomal Alu product and was negative for the Y chromosome–specific DYZ1 marker, while male control DNA was positive for both Alu and DYZ1. DNA from the first slide preparation

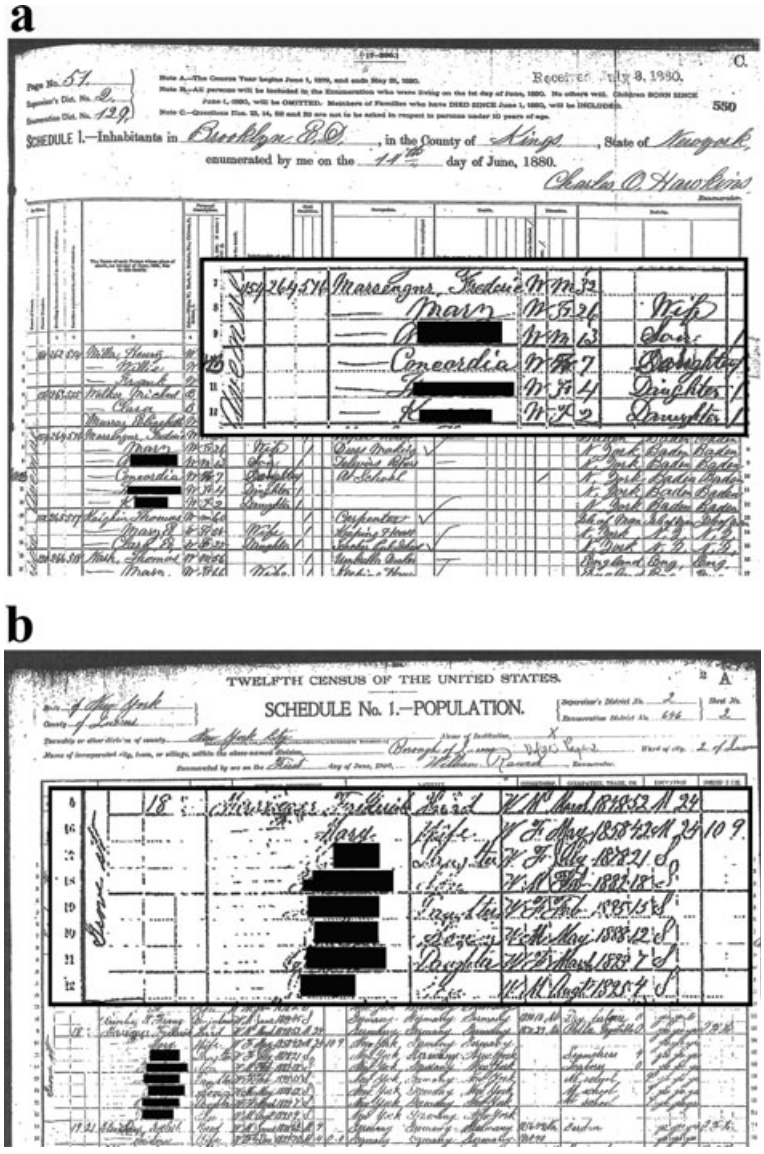


FIG. 3—*The 1880 and 1900 U.S. census records for the Mersinger family of Brooklyn, NY. Insets show details. In 1880 (a) Cora's (Concordia) parents Frederic and Mary are listed, as are brother A and sisters T and K. In the 1900 census (b), Cora's parents, and siblings F, Ju, and Jo (males), and L and B (females) are noted. Full names of Cora's siblings have been redacted for privacy considerations of living descendants.*

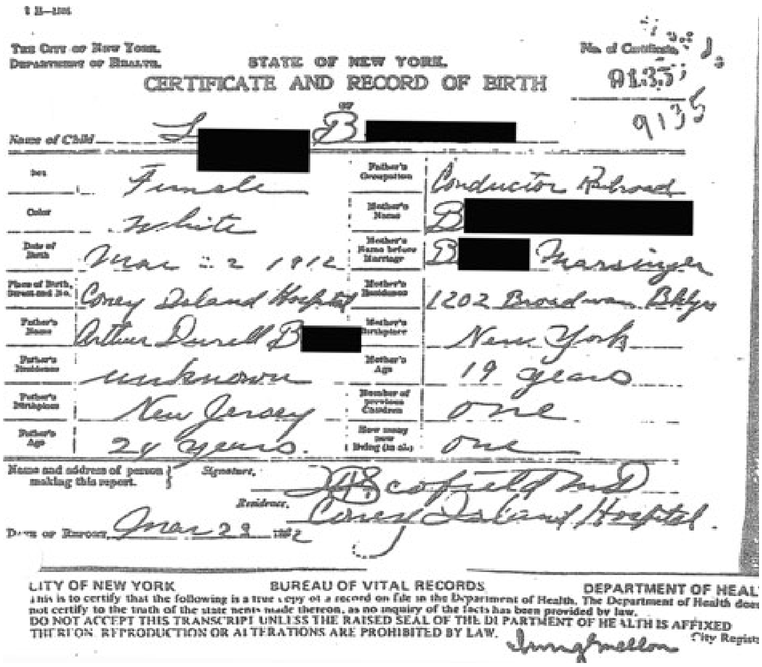
produced clear amplification curves for both Alu and DYZ1, for neat and diluted DNA (exemplified in Fig. 5, upper panel). When the tests were repeated on the second DNA purification from the slide, again both the autosomal and Y chromosome products were clearly present (Fig. 5, lower panel), denoting, in all cases, male DNA from the fixed tissue.

Discussion

The chase, capture, and trial of Dr. Crippen were uniquely sensational for their time, being followed and largely driven by the mass media. Owing to wireless communication, news of the events spread almost instantly to both sides of the Atlantic. Dr. Crippen first learned that he was being sought through newspaper reports he saw on the European mainland after leaving London and altered his and Miss LeNeve's travels by booking an earlier ship to North America. Still, the case was already so widely publicized that Captain Kendall recognized the pair was on board, and Dr. Crippen was said to have expressed relief when Inspector Dew intercepted him in Canada, after which he willingly returned to England, again, always proclaiming his innocence. The multifaceted research presented here directly speaks to that claim and may very well bear it out.

Identifying extant maternal relatives of Cora Crippen for this study was a 5-year ordeal, requiring evaluation of civil, religious, and personal documentation of the family as a unit, as well as sharing information with other genealogists. In the end, several Mersinger descendants were located, some of whom had stark memories of Cora, and many others who had had the story passed down to them. Multiple family members possessed letters, records, and photographs of Cora Crippen and her family. These searches culminated in the identification of maternal grandnieces of Cora Crippen, who themselves had memories of family stories of the British murder.

Loan of a slide (Fig. 1) used at Dr. Crippen's trial to identify the cellar remains as those of Cora Crippen meant that DNA isolation



THE CITY OF NEW YORK.
DEPARTMENT OF HEALTH.

STATE OF NEW YORK.

CERTIFICATE AND RECORD OF BIRTH

No. of Certificate 9135

Name of Child: L[redacted] B[redacted]

Sex	Female	Father's Occupation	Conductor Railroad
Color	White	Mother's Name	B[redacted]
Date of Birth	Mar 22 1912	Mother's Name before Marriage	B[redacted] Mersinger
Place of Birth, Street and No.	Coney Island Hospital	Mother's Residence	1202 Broadway Bklyn
Father's Name	Arthur Durell B[redacted]	Mother's Birthplace	New York
Father's Residence	unknown	Mother's Age	19 years
Father's Birthplace	New Jersey	Number of previous Children	one
Father's Age	24 years.	How many now living (in all)	one

Name and address of person making this report. Signature, [illegible] M.D.
Residence, Coney Island Hospital.

Date of Report, Mar 23 1912

CITY OF NEW YORK BUREAU OF VITAL RECORDS DEPARTMENT OF HEAL[TH]
This is to certify that the following is a true copy of a record on file in the Department of Health. The Department of Health does not certify to the truth of the statements made thereon, as no inquiry of the facts has been provided by law.
DO NOT ACCEPT THIS TRANSCRIPT UNLESS THE RAISED SEAL OF THE DEPARTMENT OF HEALTH IS AFFIXED THEREON. REPRODUCTION OR ALTERATIONS ARE PROHIBITED BY LAW.
City Regist[rar]

FIG. 4—*The birth certificate of the daughter of B Mersinger. That daughter, Cora's niece who was born in 1912 and died in 1973, had four children, two of whom were located. These two grandnieces and one of their daughters, the great-grandniece, donated buccal swabs for this study. Full names have been redacted for privacy considerations of living descendants.*

could commence. It was interesting to note that the slide's cover slip could not be removed using xylene as a solvent, which typically works well on today's mounting media. Some background investigation indicated that a popular cover slip adhesive at the time was pine resin, consistent with the yellowish appearance of the slides. This necessitated chipping away at the cover slip to expose the tissue beneath. When a portion of the cover slip was thus removed, the tissue, which adhered well to the slide, could be scraped off using a scalpel and DNA isolation was initiated.

Two methods of DNA purification were utilized, organic and Chelex extraction, both of which are well known to forensic laboratories, although the latter is used less frequently today. It was not clear whether one would yield superior results; however, earlier research indicated they generate different outcomes on fixed tissues (18,19), with Chelex purification potentially more likely to result in successful PCR amplification (18). Similar results were obtained in the current study, in that only DNA purified using Chelex produced amplification products. The specific reason for this is unknown, although it seems likely that it results from the cross-linking activity of formaldehyde. Formaldehyde and similar fixatives preserve tissue by cross-linking proteins (and other organics) to one another, as well as DNA to proteins (29–31). Once this occurs, DNA may very well be drawn into the organic phase during extraction and thereby discarded. In contrast, a Chelex preparation preserves all the organic molecules, leaving DNA, even if cross-linked, available for PCR. The current and previous findings are consistent with this notion and help to explain the differing amplification success obtained using the two methods.

Interestingly, the fact that only DNA from the Chelex extraction amplified is informative in and of itself. Given the historical and thus unknown nature of how the cellar tissues were dealt with at the time, how might one be assured that the DNA results obtained in this study resulted from the slide tissue, and not, for instance, from Dr. Spilsbury, who obviously handled the remains (whether he wore gloves of some kind and took other precautions is not mentioned in the records). Here, the clearly different results obtained from the two DNA extraction procedures are key, in that the standard organic extraction generated no PCR results at all, even though it yields far cleaner and more concentrated DNA than does a Chelex extraction. The amplification success from only the Chelex preparation, which is consistent with fixed tissue and not touch DNA (the latter being routinely obtained through organic extraction, as is standard in our laboratory for instance), indicates a fixed tissue origin of the DNA. Further, the slide afforded a relatively large amount of tissue, certainly many orders of magnitude more than would be present in a touch sample, particularly one dating to 1910. And there is no doubt about the integrity of the slide tissue since that time, as the cover slip, as noted earlier, was extremely secure. These, along with the copious controls incorporated and repetition of experiments, show that the mtDNA and sexing results originated from the slide tissue.

The mitochondrial haplotypes generated from the grandnieces and great-grandniece of Cora Crippen were very distinctive, containing multiple sites that differed from the reference sequence, and were unique in the FBI database. This made comparison to the slide tissue straightforward, as small mtDNA regions could be targeted from the degraded 1910 DNA. The mtDNA obtained from the Spilsbury slide was clearly not the same as Cora Crippen's grandnieces, differing at five nucleotides at a minimum. Likewise, the slide DNA sexing results were clear and highly replicable, consistently producing both autosome and Y chromosome products, revealing that the tissue originated from a man.

Based on the genealogical and molecular data presented here, only one conclusion can be drawn: the remains obtained from the Crippen's cellar at 39 Hilldrop Crescent, London, in 1910 were not those of Dr. Crippen's wife. It is beyond the scope of this article to speculate on whose they were (clearly they were human) or how they came to be in the Crippen's coal cellar. It must be noted that the forensic tools available today are far advanced from those available in 1910, so it is perhaps not surprising that new (and

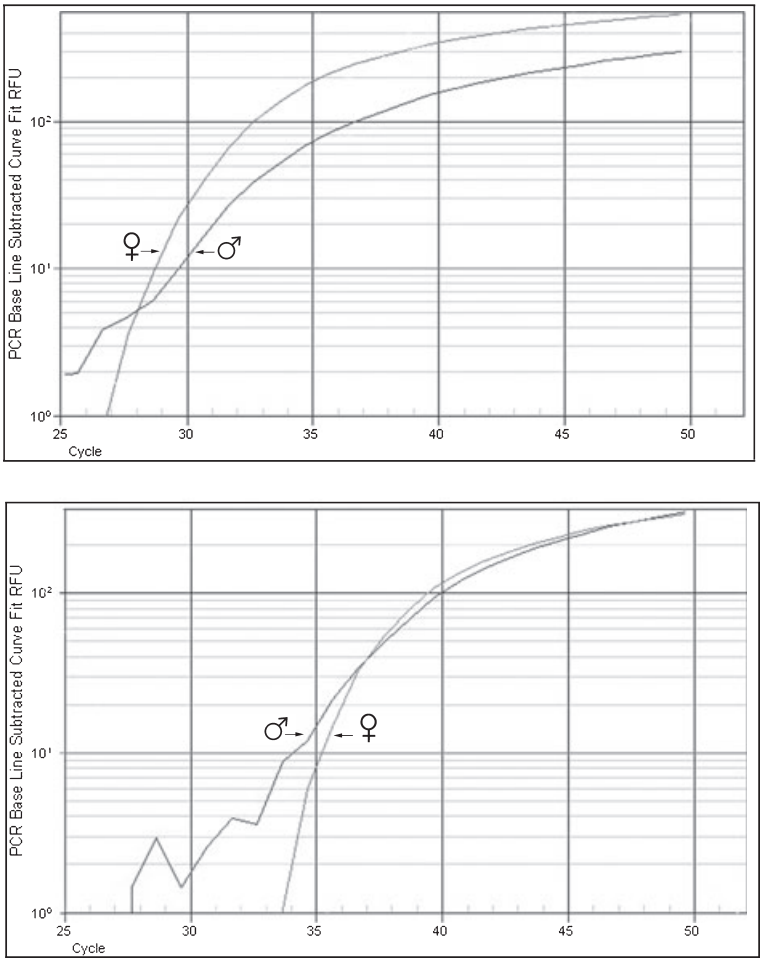


FIG. 5—*Examples of the Spilsbury slide sexing assay results using real-time PCR. The upper panel is from the first slide DNA isolation, while the lower panel is from the second DNA isolation. The x-axes show PCR cycle number; the y-axes show relative fluorescent units (RFU; with the baseline subtracted). A critical threshold value of 10 RFU was utilized. The male (Y chromosome) and "female" (Alu autosomal) curves are denoted, both clearly crossing the threshold. PCRs were run in triplicate. Negative control and reagent blanks did not amplify.*

conflicting) results were obtained, as still happens (32). Forensic science in 1910 was in its infancy, and scientists at the time had to rely on the tools and techniques available to them. DNA testing of remains, such as those found in the Crippen's cellar, would today be virtually automatic, producing far more objective results for personal identification than interpretation of small physical abnormalities in highly decayed flesh.

Finally, in light of the data presented here, we can briefly look at the outcome of Dr. Crippen's investigation and trial. As noted earlier, Dr. Crippen proclaimed his innocence throughout, stating before his hanging that "I insist I am innocent…some day evidence will be discovered to prove it…" The heinous crime for which Dr. Crippen was hanged, which intrigued much of the world in 1910, was illogical in many ways. If Dr. Crippen, described as very mild mannered, had murdered his wife, why did he openly flaunt her absence by selling many of her possessions, and taking his mistress out socially where she sometimes wore his missing wife's jewelry? Even more perplexing is the manner in which the body was discarded. If a murderer was successful in killing his victim unwitnessed, then dismembering and disposing of the head, arms, legs, and every bone, why go through the ordeal of carefully sectioning out the victim's viscera (performed in a single piece with reported surgical skill), and burying these soft tissues, excluding anything that could identify sex, in one's very own basement, along with a small amount of hair and a pajama top? It is these acts (and others) that have long led historical investigators to wonder whether Dr. Crippen actually did murder his wife and whether the cellar remains were hers.

The judge, Lord Richard Alverston, condemned Dr. Crippen to die by hanging. Before the jury's deliberation, he stated regarding the remains: "Gentlemen, I think I may pass for the purpose of your consideration from the question of whether it was a man or woman. Of course, if it was a man, again the defendant is entitled to walk out of that dock." Sexing the remains was impossible at the time, as were other purely objective methods for their identification. We are thus left with an instance of historical misidentification. Based on the genealogical and genetic investigations presented here, the remains found in Dr. Crippen's coal cellar were not only not Cora Crippen's, they were not even female.

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