

CASE REPORT

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PATHOLOGY/BIOLOGY

María de Lourdes Chávez-Briones,^{1,2} M.D.; Raquel Hernández-Cortés,² B.S.; Porfirio Díaz-Torres,² B.S.; Alberto Niderhauser-García,¹ M.D.; Jesús Ancer-Rodríguez,¹ M.D.; Gilberto Jaramillo-Rangel,¹ Ph.D.; and Marta Ortega-Martínez,¹ Ph.D.

Identification of Human Remains by DNA Analysis of the Gastrointestinal Contents of Fly Larvae

ABSTRACT: Dipterous fly larvae (maggots) are frequently collected from a corpse during a criminal investigation. Previous studies showed that DNA analysis of the gastrointestinal contents of maggots might be used to reveal the identity of a victim. However, this approach has not been used to date in legal investigations, and thus its practical usefulness is unknown. A badly burned body was discovered with its face and neck colonized by fly larvae. Given the condition of the body, identification was not possible. Short tandem repeat (STR) typing was performed using the gastrointestinal contents of maggots collected from the victim and was compared to STR profiles obtained from the alleged father. The probability of paternity was 99.685%. Thus, this comparative DNA test enabled the conclusive identification of the remains. This is the first reported case of analysis of human DNA isolated from the gastrointestinal tract of maggots used to identify a victim in a criminal case.

KEYWORDS: forensic science, forensic entomology, DNA typing, human remains, fly larvae, victim identification

Forensic entomology is the analysis of insect evidence for forensic and legal purposes (1). The most common application of entomological evidence in criminal investigations is to determine the postmortem interval, that is, the time between the death and discovery of a corpse (2). In addition, entomotoxicology studies analyzed the application of toxicological analysis on insects present on human remains in the absence of tissues and fluids normally taken for such purposes (3). Finally, a forensic entomological investigation can benefit from a variety of widely practiced molecular genotyping methods. The most commonly used is DNA-based specimen identification. Other applications include the characterization of the population genetic structure of a forensically important insect species and the identification of insect gut contents (4).

Forensic genetic analysis of insect gut contents has several potential uses (5). Dipterous fly larvae (also called maggots) can be a suitable source of human DNA. Wells et al. (6) sequenced human mitochondrial DNA from the gut contents of maggots raised on human tissue. Also, Zehner et al. (7) analyzed nuclear short tandem repeat (STR) loci of human DNA coming from maggots fed on decomposing bodies. Thus, theoretically, typing

of human DNA coming from the gastrointestinal tract of maggots and their comparison with existing genetic profiles might help reveal the identity of a victim.

However, the studies previously referred were conducted under controlled working conditions; this approach has not been used to date in legal investigations, and thus its practical usefulness is unknown. Here, we report the first case of analysis of human DNA isolated from the gastrointestinal tract of maggots used to identify a victim in a criminal case. Also, this is the first reported case of application of forensic entomology in Mexico.

Case Report

A badly burned body was discovered in a wooded area adjacent to a remote country road. The corpse was in a pugilistic posture, with the feet and hands missing. The face and neck were extensively colonized by fly larvae. Given the physical condition of the body, investigators were unable to determine either gender or approximate age.

At the scene, there was a burned patch in the soil under and around the body. No physical evidence was found near the remains, except for a school graduation ring. The body was taken to the coroner's office for an autopsy, which revealed an open head injury. Because of the state of the corpse, soft tissues were not available for further analysis, with the exception of a small burned fragment of liver. Three maggots were collected from the victim and were identified by morphologic observation as specimens of blowflies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae) (8). All of the maggots were individually

¹Departamento de Patología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Ave. Madero y Dr. Aguirre Pequeño, Colonia Mitras Centro, Monterrey, Nuevo León, México CP 64460.

²Laboratorio de Genética Forense, Dirección de Criminalística y Servicios Periciales, Agencia Estatal de Investigaciones, Procuraduría General de Justicia del Estado de Nuevo León, Ave. Gonzalitos 452 Sur, Residencial San Jerónimo, Monterrey, Nuevo León, México CP 64649.

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separated into sterile 1.5-mL microcentrifuge tubes and were preserved in 70% ethanol at 4°C.

A woman was reported missing by her father, who claimed that his daughter was abducted by several subjects 10 days earlier than the remains were found. This man recognized the school graduation ring recovered at the scene as belonging to his daughter. However, the body was so badly burned that he was unable to identify it by facial or other physical features. Several attempts to obtain a genetic profile from the fragment of liver recovered at autopsy were unsuccessful. We hypothesized that it would be possible to identify the body by the genetic analysis of the gut contents of maggots.

Each maggot was processed separately. Each larva was washed and dissected using methods previously described (9) to remove the crop, a diverticulum of the cephalic end of the gut. DNA extraction from the crops was performed using standard phenol-chloroform extraction and ethanol precipitation (10). DNA of the alleged father was extracted from a buccal sample using a Chelex protocol (11).

Polymerase chain reaction was carried out with DNA extracts from the crop contents and the buccal sample using the commercially available multiplex kit AmpF ℓ STR $^{\circledR}$ Identifier (Applied Biosystems, Foster City, CA) according to the manufacturer's recommended protocol. Capillary electrophoresis was performed in an ABI PRISM $^{\circledR}$ 310 genetic analyzer (Applied Biosystems). Samples were run on a capillary containing POP-4 polymer, the GeneScan $^{\text{TM}}$ -500 LIZ $^{\circledR}$ standard was used for sizing alleles, and data were analyzed with GeneMapper $^{\circledR}$ ID analysis software version 3.2 (Applied Biosystems). The probability of paternity was calculated using Mexican population data on STR allele frequencies (12,13) and the PATPCR software version 2.0.2 (14,15).

The genetic profiles that were generated from the samples are presented in Table 1. The same results were obtained from the three different maggots. The amelogenin locus revealed that the maggots were raised on the remains of a female person. Further, these samples gave rise to 12 loci, each of which shared at least one allele with the corresponding STR loci generated from the alleged father. The probability of paternity was 99.685%. Paternity is presumed when genetic tests establish a probability of paternity of 99% or greater (16). Thus, the comparative DNA test employed enabled the conclusive identification of the

remains. The results were later verified by the DNA analysis of bone coming from the remains. This procedure was possible only after many attempts.

Discussion

Given the accuracy and reliability of the technique, DNA genotyping is widely used in the field of forensics to identify human remains. The ideal source material for genotyping should not only be abundant and easy to collect, but also allow the generation of good-quality DNA extracts. However, in victims whose bodies underwent significant trauma or burning it is sometimes difficult to obtain biologic material suitable for genetic analysis. In this article, we report the identification of a badly burned body by means of the genetic analysis of the gastrointestinal contents of maggots raised therein and their comparison with genetic profiles of a presumptive relative.

STR profiles obtained from the maggots were incomplete. However, the number of loci successfully amplified was sufficient to perform a comparative DNA test against the alleged father, which was adequate for conclusive identification of the remains. There are many factors that can influence the success of DNA typing results. The main factors involved in human DNA analysis coming from maggots have been previously explored, and guidelines have been developed for the proper application of such procedure (1,2,4-7,9,17). We followed these guidelines as closely as possible, although according to Linville et al. (17) the best method to preserve maggots before their analysis is to store them without any fluid at -70°C, whereas we stored the maggots in 70% ethanol at 4°C. However, complete STR profiles could be obtained from maggots even after 2 months of storage in 70% ethanol (7) confirming the fact that ethanol is a useful preservative for tissue that has to be analyzed for DNA (7,18,19).

Thus, it is possible that the quality of DNA extracted from maggots was in function of the state of decomposition of the remains. This assumption can be based on the fact that each maggot was processed separately, and the same results were obtained from each of them. Furthermore, the taxon of the maggots, which are analyzed for STR typing, appears to have no influence on the results. Complete STR profiles could be obtained regardless of the Diptera family (7).

In future application of this method, we recommend following established guidelines and recognize that severe degradation of a body may not permit identification by the method.

On the other hand, to date, there are no reports about the application of forensic entomology in legal investigations in Mexico. It is necessary that our judges, prosecutors, and law enforcement agents consider entomological data as evidences applicable in solving criminal cases.

In conclusion, this is the first reported case of analysis of human DNA isolated from the gastrointestinal tract of dipterous fly larvae (maggots) used to identify a victim in a criminal case. If maggots are encountered in association with human remains, investigators could utilize this approach if the extraction of DNA from other sources is not possible.

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TABLE 1—Comparison of short tandem repeat results of DNA recovered from larvae and the alleged father of the victim.*

Locus	Victim	Alleged Father
Amelogenin	XX	XY
D8S1179	13/15	13/15
D21S11	29/30.2	30.2/33.2
D7S820	10/10	10/11
CSF1PO	n/r [†]	10/11
D3S1358	14/17	16/17
TH01	7/7	7/9.3
D13S317	12/13	9/13
D16S539	10/11	11/12
D2S1338	24/25	17/25
D19S433	15/16	13/15
vWA	16/18	15/16
TPOX	8/8	8/8
D18S51	n/r	14/15
D5S818	12/12	11/12
FGA	n/r	18/26

*Matching alleles are marked in bold.

[†]No result was obtained.

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Additional information and reprint requests:
 Marta Ortega-Martínez, Ph.D.
 Departamento de Patología, Facultad de Medicina
 Universidad Autónoma de Nuevo León
 Ave. Madero y Dr. Aguirre Pequeño, Colonia Mitras Centro
 Monterrey, Nuevo León
 México CP 64460
 E-mail: martha.ortegam@uanl.mx