TECHNICAL NOTE

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Human and Insect Mitochondrial DNA Analysis from Maggots*

REFERENCE: Wells JD, Introna F, Jr, Di Vella G, Campobasso CP, Hayes J, Sperling FAH. Human and insect mitochondrial DNA analysis from maggots. J Forensic Sci 2001;46(3):685–687.

ABSTRACT: During the course of our forensic investigations, we have encountered situations where it would have been useful to have evidence, other than direct contact between the two, for concluding that a carrion-fly maggot developed on a particular human victim. If a maggot collected during a death investigation did not develop on the victim, then its age is not relevant to estimating the postmortem interval. In this study we demonstrate that mitochondrial DNA (mtDNA) sequence data can be obtained from the dissected gut of a maggot that had fed on human tissue. These data can be used to identify both the human corpse upon which the maggot had been feeding and the species of the maggot itself.

KEYWORDS: forensic science, forensic entomology, mitochondrial DNA, cytochrome oxidase, hypervariable region, species identification, death investigation, *Cynomyopsis cadaverina*

The most common use for insect evidence collected during a death investigation is in estimating the postmortem interval (PMI). When this estimate is based on the age of a maggot (fly larva), it is assumed that all of the maggot's development and feeding occurred on the victim, because only then is the age of the larva relevant to PMI. In the typical case this assumption is justified because the larva was collected directly from or near a corpse that shows other signs of decomposition. However, such a direct and certain association cannot always be made, and during the course of our casework we have encountered a variety of situations where an alternative method for associating a maggot with a corpse would have been useful to us.

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* Presented at the American Academy of Forensic Sciences 1999 Annual Meeting. This work was supported by a University of Alabama at Birmingham Faculty Research grant to J.D.W., and by National Institute of Justice grants 97-IJ-CX-0035 to F.A.H.S. and 99-IJ-CX-0034 to J.D.W. The views expressed here are not necessarily those of the U.S. Department of Justice.
Received 2 May 2000; accepted 5 July 2000.

Case Summary and Profiling Results

Situation 1: Investigators Discover Maggots but No Corpse

The presence of live maggots in the absence of a dead body at a location is almost certain evidence that some kind of corpse has been removed from the scene. If it had been an animal carcass, then it could have been removed by vertebrate scavengers or disposed of by a person without any crime having been committed. Obviously, if it had been a human corpse, then further legal investigation would be warranted.

Example—In Brindisi County (southern Italy) a police informant identified a cellar in an isolated farmhouse where a murder had occurred and the body had been left. It was later learned that the criminal organization responsible for the murder learned of this "betrayal" and removed the body in an effort to discredit the informant. Police investigators recovered only a large number of *Chrysomya albiceps* larvae, and no other biological evidence, from the cellar.

Situation 2: Maggots are Not Found Directly on a Corpse, and an Alternative Food Source is Nearby

Larvae may crawl from a corpse if they are physically disturbed, if the soft tissue (food) is exhausted before they finish eating, or if they reach full size and enter the postfeeding stage. If more than one potential food source is found close to such a larva, it may not be obvious from which the larva originated.

Example—The body of an infant with attached placenta was discovered in the household garbage being transferred from one municipal truck to another near Demopolis, AL. The body was received at the medical examiner's office, along with the material that had surrounded it at the time of discovery, and this included a large amount of moist dog food. *Sarcophaga bullata* larvae were found crawling throughout the material that was brought in. It was not clear whether the larvae originated from the dog food or from the infant.

Situation 3: Maggots are Found on a Corpse, but May Have Come from Somewhere Else

Carrion-fly larvae are limited in their ability to crawl compared to most insects (1,2), although postfeeding individuals of some

species can move several meters away from the food when seeking a place to pupate (3).

Example—The body of an adult female was discovered in a burning house in Berkeley, CA. The body had been partially burnt, making it difficult for the medical examiner to estimate either the time or cause of death. Nearly full-grown sarcophagid larvae were collected from the victim. The larvae could not be identified to the species level. However, given the species of carrion-feeding sarcophagids found in Berkeley, they were at least two days old, and if temperatures in the house were similar to those outside, then the larvae were probably four days old. Therefore the victim was almost certainly dead before the fire started. This contradicted the accused person's claim to have seen the victim alive only a few hours before the fire. During the trial, the defense attorney argued that the larvae, disturbed by the fire, could have crawled to the victim's body from some unknown source, such as a dead rodent in the wall.

In this case the accused was convicted of murder, suggesting that the jury did not believe that the maggots on the victim came from a mysterious alternate source. Nevertheless, we suspect that situations do occur in which a plausible alternate maggot food source is found quite close to a human corpse on which maggots are feeding.

It has been shown that human DNA from the gut of a blood-feeding insect can be matched with the original human source (4,5). Maggot gut contents present similar material for associating an insect and its last source of food.

Because we are also engaged in a research program to develop DNA-based identification methods for forensically important insects (6), it would suit our purposes to use the same DNA extract for the dual purpose of identifying a maggot and associating it with a particular victim. In this study we extracted and identified the source of mitochondrial DNA (mtDNA) from both the gut contents and the gut itself from maggots fed on human tissue.

Material and Methods

Insect Samples

Eggs were obtained from wild flies near San Antonio, TX. Newly hatched larvae were transferred to a rearing jar containing tissue from a human liver that had been removed from a transplant recipient. Third larval instars approximately 12 mm in length were preserved in 70% ethanol and stored at −20°C. Some larvae were allowed to pupate and emerge as adults. These were identified as the bluebottle fly *Cynomyopsis cadaverina.*

Each of the larvae was dissected and the crop, a diverticulum of the anterior end of the gut, was removed. Dissected crops were approximately 3 mm in length.

Reference Samples of Human and Fly DNA

Blood donated by the same individual who was the source of the liver tissue, and thoracic muscle from one of the emerged adult flies, were used to produce reference haplotypes to be compared to human and fly DNA from the maggot crops.

DNA Methods

The DNA of each crop and its contents, as well as the adult fly, was extracted using QIAamp® columns (Vallencia, CA) following the manufacturer's tissue protocol. The blood reference specimen was similarly processed using the whole blood protocol. In order to avoid the remote possibility of contamination of crop extracts by the more rich source of DNA represented by the reference specimens, the latter were processed after all laboratory analyses of the former were complete.

Each DNA extract was used as template for two PCR reactions. A region of the fly cytochrome oxidase subunit one (COI) was amplified using primers C1-J-1751 and C1-N-2191 (7), and a region of the human hypervariable region 2 (HV2) was amplified using primers F155 (5′ TATTTATCGCACCTACGTTC 3′), suggested by members of the Armed Forces Institute of Pathology, and R340 (8). The forward-strand sequence of each PCR product was determined using a PE-Biosystems (Foster City, CA) 310 genetic analyzer and the BigDye Terminator® sequencing kit, following manufacturer's instructions.

Computer Analyses and Software

Sequences were confirmed and aligned manually using Sequence Navigator (PE-Biosystems).

Ethical Requirements

The University of Alabama at Birmingham Institutional Review Board For Human Use approved all procedures for sampling and analysis of human tissue.

Results

Insect mtDNA

A fragment of COI was successfully amplified from all five larval crops and the adult fly. All amplicons yielded sequence data for the entire region between the two primers. As expected, the COI primers failed to amplify DNA from the reference blood sample. The sequence for the adult fly has been deposited in GenBank (accession number AF259505, Fig. 1). Two of the larvae produced COI haplotypes identical to the adult fly, and three differed from the adult by the substitution of a thymine for an adenine at position

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          .         .         .         .         .         .         .
TCGGATAAACAATATAAGTTTCTGACTTTTACCTCCTGCATTAACTTTACTATTAGTAAGTAGTATAGTGGAAAACGGAG     1831
          .         .         .         .         .         .         .
CTGGAACAGGATGAACTGTTTACCCACCTTTATCATCTAATATCGCTCATGGAGGAGCTTCTGTTGATTTAGCTATTTTT     1911
          .         .         .         .         .         .         .
TCTTTACACTTAGCAGGAATTTCTTCAATTTTAGGAGCTGTAAATTTTATTACAACAGTTATTAATATACGATCAACAGG     1991
          .         .         .         .         .         .         .
AATTACTTTTGACCGAATACCACTATTCGTTTGATCAGTAGTAATTACAGCTTTATTACTTTTACTATCTTTACCTGTTC     2071
          .         .         .         .         .         .         .
TAGCTGGTGCTATTACAATATTATTAACAGACCGAAACCTTAATACTTCATTCTTCGACCCAGCAGGAGGACGAGACCCA     2151
          .         .         .
ATTCTATACCAACATTTATTTTGATTTTTTGGTCACCCT                                           2190
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FIG. 1—*Mitochondrial DNA sequence of a portion of the gene for cytochrome oxidase subunit one from* Cynomyopsis cadaverina*. Position numbers correspond to those for* Drosophila yakuba *(23).*

2058 (Fig. 1). This is a silent substitution, resulting in no change in the amino acid sequence, and is typical of the COI haplotype diversity we have found within carrion-fly species (7,9).

Human mtDNA

A fragment of HV2 was successfully amplified from three of five crop extracts and from the blood reference sample. As expected the HV2 primers failed to amplify DNA from the reference adult fly. Sequence data corresponding to base positions 206–321 of the standard human mtDNA sequence (GenBank accession number M12548), as well as the R340 primer sequence, were obtained and aligned for all PCR amplicons. All HV2 sequences were identical and differed from the standard human sequence by the presence of a guanine at position 263 and by a cytosine at position 315.1. Electropherograms for the crop extracts were less easy to read at the 5′ end compared to the blood sample, which produced readable sequence data beginning at position 180.

Clearly, maggot crops can be a suitable source of DNA for identification of both the insect and its gut contents.

Discussion

The forensic value of mtDNA for identifying individual human remains (10) and both vertebrate (11,12) and insect species (7,13,14) has been repeatedly demonstrated. A considerable amount of research effort has been focussed on the development and validation of new analytical techniques (15,16), and on expanding the list of biological tissues shown to yield mtDNA that is suitable for typing (e.g., 4,8,11,17–20).

In this study we have further expanded the list of sources of DNA evidence by showing that mtDNA analysis may potentially be used to associate a maggot with a human corpse, even if physical contact between the two is not observed.

We suggest that several avenues of research be pursued in order to refine this technique. Recommended field collection methods should be defined. Carrion larvae collected during a forensic investigation may be preserved using a wide variety of fluids (21). The effect that these preservatives have on subsequent DNA extraction and PCR should be evaluated, so that investigators will not unknowingly render samples unsuitable for genetic analysis. It may be possible to streamline the laboratory procedure by performing a duplex PCR reaction of human and fly DNA (see 22), and an attempt should be made to amplify nuclear loci from similar samples. Finally, although the analysis presented here can identify a maggot's "last meal," we do not know how long a maggot can cease feeding before gut-content DNA cannot be recovered. It is also true that these analyses cannot eliminate the possibility that a maggot fed on a completely different food source at some earlier point in its development. Therefore, studies should be undertaken to investigate the turnover and degradation of host DNA after a maggot is removed from food or is placed on a new food type.

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