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TECHNICAL NOTE

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Mitochondrial DNA Sequencing of Beetle Larvae (*Nitidulidae: Omosita*) Recovered From Human Bone*

ABSTRACT: The isolation, amplification, and characterization of human DNA from hematophagous (blood feeding) and necrophagous (carrion feeding) arthropods have been advanced significantly by the development of polymerase chain reaction (PCR) DNA sequencing methodologies. Historically, DNA technology has been successfully utilized to identify individual hosts upon which species of hematophagous arthropods have fed. The analysis of hematophagous insects' gut content blood meals has led to major advances in medical entomology and vector-borne disease epidemiology. In the forensic arena, the ability to apply similar techniques to insects recovered from badly decomposed remains has been greatly enhanced through the advent of mitochondrial DNA (mtDNA) techniques. Mitochondrial DNA analyses have been utilized to identify both the human remains upon which fly larvae (maggots) have fed and the species of the larvae themselves. The preliminary work detailed here demonstrates, for the first time, the successful application of mtDNA sequencing techniques to the analysis of necrophagous beetle larvae. A small sample of sap beetle larvae, *Omosita spp.* (*Coleoptera: Nitidulidae*), was collected from human skeletal remains during anthropological examination and analyzed for human DNA using mtDNA sequencing. The beetle larvae yielded mtDNA matching that of the host human bone. The results detailed here further demonstrate the robust nature of human mtDNA and the ability to recover valuable mtDNA evidence from forensically important, late decompositional stage insect species.

KEYWORDS: forensic science, mitochondrial DNA, polymerase chain reaction, decomposition, *Nitidulidae*, *Omosita*, sap beetle, skeletal remains

DNA technology has given researchers the ability to isolate, amplify, and analyze minute quantities of human DNA. Forensically, the traditional use of such capabilities has been to identify victims and suspects using blood or other body fluids. Entomologically, polymerase chain reaction (PCR) DNA techniques have been successfully used to extract host DNA from hematophagous arthropod blood meals recovered from the gut contents of a variety of blood-feeding insects (1). Studies by Gokool and Coulson et al., for example, have successfully identified individual hosts from mosquito (*Anopheles gambiae*) blood meals utilizing PCR (2,3). The forensic value of identifying individual human remains and both vertebrate and insect species has been repeatedly demonstrated (4–6). Introna et al. have successfully recovered both human and animal host DNA from the crops of necrophagous fly larvae (maggots) fed on decomposing tissues (7). PCR has also proven useful in identifying and differentiating between species of forensically important *Diptera* (4,8,9).

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Little attention has been given to the potential for host DNA recovery from adult or immature insects that colonize and feed on remains in the later stages of decomposition. These arthropods are frequently encountered on badly decomposed and skeletalized remains and in the vicinity of where such remains are discovered.

PCR technology and mitochondrial mtDNA techniques offer forensic scientists the capability of analyzing genetic polymorphisms in skeletal and advanced decompositional remains. To assess the applicability of mtDNA techniques to the analysis of late decompositional stage carrion insects, we conducted mtDNA testing on larval sap beetles, *Omosita spp.* (*Coleoptera Nitidulidae*), recovered from human bones. The larvae of *Omosita spp.* are common, late-stage carrion inhabitants that feed on caseic and saponified tissues. The bones from which the larvae were recovered had been subjected to environmental exposure for several months. This paper describes the extraction, quantification, and typing of human mtDNA from whole beetle larvae.

Materials and Methods

Human bones that had been exposed to the environment for several months were harvested in an attempt to characterize mtDNA for identification purposes. Attached to a rib bone were numerous beetle larvae that, it was presumed, had been feeding upon the skeletalized remains at the time that the bone was harvested. These larvae were subsequently identified as *Omosita spp.* (*Coleoptera:*

FIG. 1—*Photomicrograph of the larva of Omosita spp. (Coleoptera: Nitidulidae). Omosita larvae are common, late-stage carrion inhabitants that feed on caseous and saponified tissues often in association with Dermestid beetles.*

Nitidulidae) (sap beetles). As previously mentioned, *Omosita spp.* (Fig. 1) are common, late-stage carrion inhabitants that feed on caseous and saponified tissues often in association with Dermestid beetles. Shortly after being received in the laboratory, the bone was placed in a freezer. The larvae were frozen along with the bone sample.

A limited, but representative, sub-sample of the collected larvae was selected for mtDNA analysis. Additional larvae were preserved for future study. The larvae and bone were separated and subsequently subjected to the same extraction procedure. A known blood sample for the skeletal remains was also provided for separate mtDNA analysis.

DNA Extraction

Bone submitted for identification was cut using a dremel tool with separating disk to a piece measuring approximately 1 by 2 in. (2.54 to 5.08 cm) The intact bone was sanded with a dremel tool equipped with an emory disk. The outer surface of the bone was sanded to remove all dirt and debris. The sanded bone was then placed in a Bessman tissue pulverizer and struck until the bone became soft and malleable. Utilizing a clean scalpel, the crushed bone was cut into small pieces. Approximately 0.02 to 0.05 g of cut, pulverized bone was placed into a plastic 1.5-mL tube containing 300 μL of stain extraction buffer (SEB). SEB contains 7.5 μL of Proteinase K (20 mg/mL) and Dithiothreitol (DTT) (30 mg/5 mL SEB).

Whole sap beetle larvae were prepared for DNA extraction by initially washing them repeatedly in a 70% ETOH solution. The larvae were then sectioned along the dorsal midline. Larval specimens were suspended in 300 μL of SEB. Replicates of both single larvae and pooled specimens (X3) were analyzed.

All samples were briefly vortexed and pulse spun in a microfuge to force fragments into the SEB. This was followed by a 2-h incubation period at 56°C. Incubated samples were then treated with a solution of phenol, chloroform, and isoamyl alcohol (24:24:1) (PCIA). The treated samples were once again spun in a microfuge at high speed for 2 min.

The aqueous phases of the PCIA were then transferred to Microcon 100 microconcentrators containing 100 μL sterile, filtered Tris-EDTA buffer (10 m*M* Tris, 0.1 m*M* EDTA, pH 8.0) (TE). Microcon tubes were centrifuged at 5000 rpm for 5 min. Washes were discarded, and 100 μL of sterile, filtered TE was added to the top of the Microcon tubes. The samples were again centrifuged at 5000 rpm for 5 min. Once centrifugation was completed, the tubes were discarded, and 200 μL sterile, filtered TE was added to the upper chamber of the Microcon device. Sample recovery tubes were added to a filtration apparatus. Samples were vortexed briefly on medium speed with the sample recovery tube pointing upward. The sample recovery tubes and filter apparatus were flipped and spun at 12 000 × *g* for 3 min. Recovery volume was approximately 200 μL.

DNA Amplification and Sequencing

PCR was utilized for the amplification of human mtDNA from all samples. Amplification was performed in 25-μL reactions using a GeneAmp System 9600 thermal cycler (Perkin-Elmer). Each reaction contained 50 m*M* potassium chloride, 10 m*M* Tris-HCl pH 8.3, 1.5 m*M* magnesium chloride, 0.001% gelatin, 5.0 pmol of each primer (0.2 μ*M*), 4 μg BSA (Sigma), 200 μ*M* each dNTP, and 5 units Taq polymerase (Perkin-Elmer). Ten μL of sample extract were used in four separate PCR reactions encompassing HV1 and HV2 (10).

Following amplification, samples were cycle sequenced utilizing a Perkin-Elmer/Applied Biosystems Division (PE/ABD) Taq Dye Terminator Cycle Sequencing kit. The kit was utilized according to the manufacturer's recommendations. One exception to the procedure was the substitution of 100 m*M* ammonium chloride for 100 m*M* ammonium sulfate in the reaction buffer. After quantification by capillary electrophoresis, dilutions of each sample were utilized as a DNA template in the cycle sequencing reaction (7 μL of a 5 ng/μL sample utilized) (10). Cycle sequencing reactions were performed according to the instructions provided by the manufacturer. An automated DNA Sequencer (PE/ABD 373A) was used to conduct sequencing.

Results and Discussion

A known blood sample from the bone donor was obtained and sequenced along with the bone and beetle samples (10). Sequence analyses revealed that mtDNA from the known blood, bone, and *Omosita* larvae were identical (Fig. 2). Apparently, the mitochondria ingested by the beetle from the host tissue had not broken down sufficiently enough to degrade the mtDNA to an extent that amplification was not possible.

The ability to utilize mtDNA for analysis is a distinct advantage in forensics. While nuclear DNA is limited to only two copies per cell, there are thousands of copies of mitochondrial DNA per cell (11). Human host mtDNA has been successfully sequenced from

SAMPLE DESCRIPTION	SAMPLE NO.															
CAMBRIDGE REFERENCE SEQUENCE[14]																
BONE FROM REMAINS	4A							C			G					C
WHOLE LARVA FROM REMAINS	5A							C			G					C
SECTIONED LARVA FROM REMAINS	B1							C			G					C

FIG. 2—*Sequence confirmation for mtDNA typing of bone samples and sap beetle (Omosita:Nitidulidae)larvae obtained from human skeletal remains.*

Pthirus Pubis (*L.*)(human crab louse) blood meals (12). These research findings contribute to the expectation that mtDNA may survive late stage decompositional events and be subsequently recovered from late succession insects such as the sap beetle.

Although the sample size for this experiment is small, the results reported here indicate that it is possible to obtain mtDNA from late-stage carrion feeding beetle larvae. The continuity among samples of bone, blood, and larvae suggest that mtDNA remains sufficiently intact after ingestion by late-stage hematophagous arthropods to produce a host mtDNA profile. It remains unclear under what conditions recoverable mtDNA can be extracted from these insects. Studies by Replogle et al. have demonstrated that host DNA can be amplified by PCR from crab lice excreta (13). This suggests that with PCR and mtDNA technologies the host DNA may be retrievable throughout an insect life cycle and beyond.

The results of our study illustrate that the typing of human mtDNA derived from necrophagous beetles feeding on human remains is possible. These findings may have forensic application in the identification of advanced decompositional and skeletonized remains. Epidemiologically, these findings may further find application in the study of insect disease vectors. Medical researchers and entomologists may be able to employ similar techniques in the study of potential disease transmission by necrophagous beetles. Furthermore, the successful sequencing of human host mtDNA from *Omosita spp*. beetle larvae demonstrates the value of mtDNA-based analyses to future forensic entomology research endeavors. We hope that the research reported here will encourage further study utilizing a wide variety of necrophagous arthropods.

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