Entomological Specimens Obtained from Human Remains offer a Faster Option for DNA Identification

Alexa Harrod[1*]
[1]*Arkansas State Crime Laboratory, Office of the Medical Examiner*

*****corresponding author: alexakwai@gmail.com

ABSTRACT

Genetic identification of human remains in advanced stages of decomposition traditionally involves the extraction of DNA from a toenail, bone, or tooth. This extraction protocol typically relies on an overnight incubation and may yield a degraded, unusable DNA profile. Insect life on the remains may provide an equally viable, yet often overlooked means to obtain a DNA profile in a shorter amount of time. This study conducted at the Arkansas State Crime Laboratory (ASCL) examines the possibility of obtaining human DNA from the entomological specimens that accompanied the remains. It also examines the incubation time of the specimens and the class of insects from which a profile could be obtained. Fly and beetle larvae were initially frozen before extraction using the following instruments: Qiagen DNA Investigator Kit in conjunction with the Qiagen EZ1 robotic workstation, Qiagen Quantiplex Pro in conjunction with the Applied Biosystems 7500 Real-Time PCR Instrument, Promega PowerPlex Fusion 6C kit with an Applied Biosystems Veriti Thermal Cycler, and finally an Applied Biosystems 3500xL Genetic Analyzer. Of the fifteen samples of insect life collected, fly larva (Diptera) yielded four usable profiles. The beetle larva (Coleoptera) specimens yielded no usable profiles. Using insect-harvested DNA to identify human remains, in some cases, allows for a faster turnaround time so that remains can be released for mortuary care as quickly as possible.

Keywords: forensic entomology, STR typing, human remains, DNA profile, advanced decomposition

Introduction

DNA STR typing is the gold standard for human identification in today's crime laboratory. STR profiles are used to establish paternity, identify human remains, and match crime scene evidence to known DNA samples (3). The timely and accurate identification of human remains can be particularly challenging when remains are in advanced stages of decomposition. In these instances, a DNA profile cannot always be obtained by a blood sample and analysts must resort to extracting DNA from a toenail, tooth, or bone sample. These types of specimens require lengthy incubation times during extraction and usually take several days to obtain results. Frequently, poor results further delay proper identification. This study demonstrates forensic entomology specimens offer a faster alternative with an extraction incubation time of only 20 minutes.

Entomological specimens from various species of insects often accompany the decomposed remains which come to crime laboratories for autopsy. These specimens are easily identified and collected by morgue staff. They are readily preserved and can be stored in a freezer for long periods of time without sample degradation. Previous studies have already determined that human STR profiles can be obtained from Dipteran of various species and life stages (1, 8). Additionally, bed bugs, sand flies, mosquitoes, lice and beetles have all been the focus of studies which have produced human DNA profiles from these insects' gut contents (2, 6, 7).

This avenue of analysis shows that forensic entomology can be a useful tool for the identification of human remains in challenging circumstances. Since crime scenes by their very nature are not carefully controlled environments like most scientific laboratories are, discovering the precise time insects first appear on remains and begin collecting blood meals can be challenging. Live entomological specimens which accompany the human remains from these chaotic environments are typically viewed as a nuisance. Here we view them as an investigative resource to be studied which may simplify the process of identification.

This study examined fifteen cases with entomological specimens from fifteen different remains at various stages of decomposition. Each of these specimens was subjected to quantitative analysis and further STR testing if enough DNA was present to yield a profile.

Materials and Methods

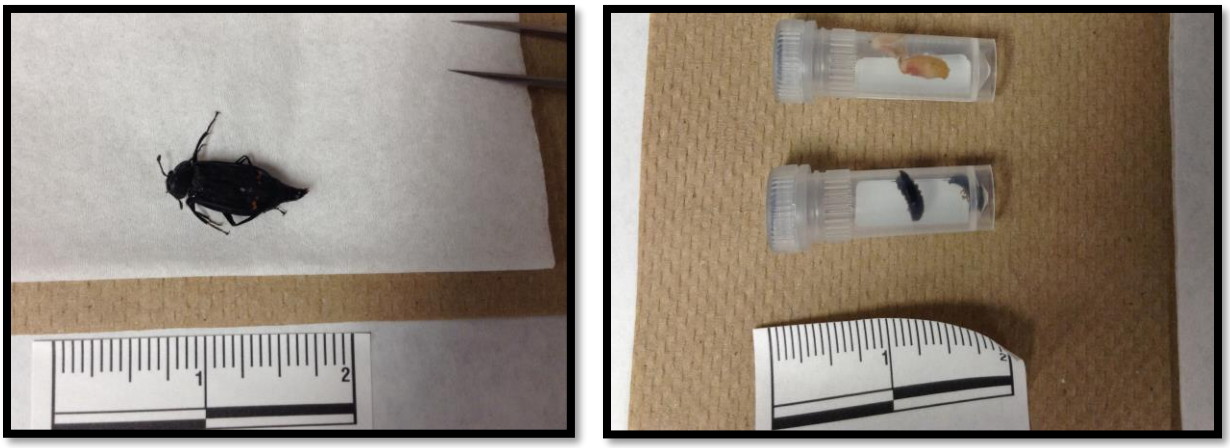
Collection of Entomological Specimens

The morgue staff identified and collected the insect life that accompanied the human remains of fifteen individuals. The samples were gathered into specimen cups labeled with the case number for corroboration that any DNA profile obtained from the study corresponded with the profile obtained by traditional methods. Each case was assigned a letter designation for deidentification purposes. All but two samples were stored in the morgue freezer. These samples were collected from June 2019 to November 2019. The remaining two samples were

collected in 2014 and 2016. Both of these samples were stored at room temperature.

Figure 1—Case D-(2019) Evidence photograph five Dipteran larvae before DNA extraction.

Figure 2- Case E- (2019) Surinam Carrion Beetle (*Necrodes surinamensis)* (4). This adult beetle, on the left, was collected in the body bag and was submitted with the beetle larvae, as seen in extraction tubes on the right.



DNA Extraction and Quantification

Each of the insect larvae was bisected laterally before being placed in a labeled extraction tube along with the section of the Kim wipe on which they were cut (Kimberly-Clark, Neenah, WI). All Qiagen reagents were used for the extraction protocol. Qiagen DNA Investigator kit extractions utilized a Qiagen EZ1 workstation (Qiagen Inc., Germantown, MD). 200ul of G2 buffer and 20ul of proteinase K were added to the specimens and incubated on a heat block for 20 minutes at 58 degrees Celsius (Qiagen Inc., Germantown, MD). An additional specimen set was run simultaneously with the first sample group. The additional set contained double the larvae and was incubated for twenty-four hours. The EZ1 workstation instrument extracts the genomic DNA using metallic bead technology and elutes the DNA into 50ul of TE buffer (Qiagen Inc., Germantown, MD). A reagent blank accompanied each batch of samples throughout the process to confirm that no contamination occurred.

Each DNA sample was quantified using the Qiagen Investigator Quantiplex Pro kit on the Applied Biosystems 7500 Real-Time PCR System to discover the amount of human DNA in the sample (Applied Biosystems, Forest City, CA). Samples that yielded 0.0025ng/ul or more of total DNA continued to the amplification process in accordance with ASCL validation guidelines. Samples which yielded less than 0.0025ng/ul of total DNA were considered too dilute to produce a usable profile.

DNA Amplification and STR typing

The samples that met the quantification standard were amplified using the Promega PowerPlex Fusion 6C system kit for STR typing with a total target amount of 1 ng of DNA (Promega Corporation, Madison, WI). They were then run on an AB 3500xL Genetic Analyzer (Applied Biosystems, Forest City, CA); the results were analyzed by Genemapper IDX (Applied Biosystems, Forest City, CA). If the sample yielded a profile, this profile was compared to the previously obtained profile to confirm the findings and to ensure no contamination occurred.

Results

This project aimed to identify an alternative DNA source for identifying human remains in advanced decomposition in a real-world crime laboratory in lieu of the current method which requires the lengthy processing of a bone, tooth or toenail. From the fifteen insect specimens that were collected from individual human remains, two yielded full STR profiles. Two yielded partial DNA profiles that were complete enough to be used for human identification. Each profile or partial profile matched the official profile for the human remains in the casefile.

Eleven specimens did not contain enough DNA to meet the ASCL's criteria for amplification, and therefore, were not run on the Genetic Analyzer (Applied Biosystems, Forest City, CA). None of the duplicate samples with the increased volume of larvae and twenty-four-hour incubation time yielded enough DNA to obtain a usable profile.

Table 1—*Results from Quantification of insect larvae removed from human remains transported to Arkansas State Crime Lab. Included are the methods of identification for each case. Blood card fail indicates that a blood sample was attempted as the first source of DNA identification, but did not produce a profile. In these cases, the analyst proceeded to DNA extraction from a toenail to generate the profile for victim identification.*

Case	*DNA profile from insect specimens*	*Total Quantity (ng/ml)*	*Insect type*	*Current ID method*	*# of larvae used*	*Specimen Notes*
A	Partial profile	0.0020	Fly Larvae/ dried	Blood Card	mass	specimen kept at room temperature for 5 years
B	Partial profile	0.0046	Fly Larvae	Toenail	4	
C	Full profile	0.1590	Fly Larvae	Blood Card fail/Toenail	4	specimen kept at room temperature for 3 years
D	Full profile	0.0075	Fly Larvae	Blood Card fail/Toenail	5	
E	No profile	0.0000	Beetle larvae	Teeth	2	only victim's head recovered due to decomposition
F	No profile	0.0002	Beetle larvae	Toenail	2	victim was deceased days before discovery in summer
G	No profile	0.0000	Fly Larvae	Blood Card	3	
H	No profile	0.0004	Fly Larvae	Prints	4	
I	No profile	0.0000	Beetle larvae	Toenail	2	victim missing almost a month, found in water
J	No profile	0.0002	Beetle larvae	Toenail	3	
K	No profile	0.0002	Fly Larvae	Toenail	2	
L	No profile	0.0007	Fly Larvae	Blood Card	5	
M	No profile	0.0001	Fly Larvae	Prints	4	
N	No profile	0.0002	Fly Larvae	Prints	7	
O	No profile	0.0000	Fly Larvae	Toenail	5	

Discussion

These results show that insect specimens can be used in practical settings to identify human remains. No additional equipment or reagents were necessary for this study. The morgue staff did not need any special training or expertise for the collection of the specimens. The limited data indicates that cases with decomposition at a point that fly larvae are present have the potential to produce a usable DNA profile. Beetles, such as the Surinam Carrion Beetle seen in figure 2, typically appear at a death site later than blowflies and their larvae, in this study, did not yield any useable profile (5). Therefore, human remains with beetles and beetle larvae present would have a greater chance of theses insects consuming degraded DNA that is less likely to be useful. Due to the fact that it is not visually evident if a blood card will yield a profile, blood and insects could be collected and processed concurrently to expedite identification.

Each identification is naturally unique in circumstance, season, and location. There is no way to account for each variable that may influence the condition that human remains arrive at our crime lab. Forensic scientists must be amenable and creative at times to provide the most accurate findings for each case. As seen on table 1, 60 percent of the 15 individuals in this study were identified by tooth or toenail. Not only do insect larvae offer a faster method of obtaining DNA from decomposed human remains, they offer a more sanitized option to DNA analysts who can experience a measure of trauma that may come from working with a tooth, toenail, or any other recognizable element of a deceased human being. Toenails are specifically troublesome specimens to process and are not always guaranteed to produce a genetic profile.

Further investigation is needed to better understand the variables which determine when entomological specimens are a viable DNA source. Additional studies could determine if beetle larvae can yield DNA profiles or if specific species of Dipteran produce more profiles than others. More in-depth studies could examine the estimated time after death that insect specimens become the best method of identification as opposed to a blood card or toenail. Also, can visual clues help steer morgue staff to choose the best specimens to yield a profile? For example, do the colors of the larvae indicate a blood meal has been taken and if so, what color produces the most complete genetic profile? Since insects are a ubiquitous presence in all environments, they offer a largely untapped resource to better our craft.

This type of study at an already accredited forensic DNA laboratory cost little in finance and time, but has great value with regard to the knowledge gained and offers the potential to serve the public in the most accurate and timely way possible.

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