Recovery of Human DNA from Maggots

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INTRODUCTION

Forensic entomology is the application of insect biology to civil and criminal law, associated with the estimation of postmortem interval (PMI) (1). Insects, such as fly larvae (commonly referred to as maggots), can be used to recover human DNA, which can undergo short-tandem repeat (STR) (2-5) and mitochondrial DNA (mtDNA) analysis (2,4,6). The alimentary canal of a fly larva includes a diverticulated crop (**Fig. 1**) that serves as a storage organ for food. Food, and therefore human DNA, is not digested in the crop and is preserved (7-8). The crop can be dissected and subjected to DNA extraction, as it may serve as a source of human DNA. Lack of an optimized extraction method for the recovery of human DNA from fly larvae may contribute to the reluctance to use this technique in the United States. Determining a proper extraction technique may lead to the use of fly larvae as a sample for human DNA. Instances in which fly larvae can be used include early decomposition, burned cadavers, or secondary scenes in which bodies have been moved to.

This study builds on research previously conducted to evaluate different extraction techniques for the recovery of host DNA from fly larvae (9). The DNeasy® Powersoil® Pro Kit was chosen based on its application to DNA extraction from insects and its purification steps which include the use of Inhibitory Removal Technology and silica column purification. Human DNA was recovered from various fly larvae sample types, and DNA extracts underwent STR genotyping, mtDNA analysis, MainstAY, and DNA barcoding of the fly larvae.

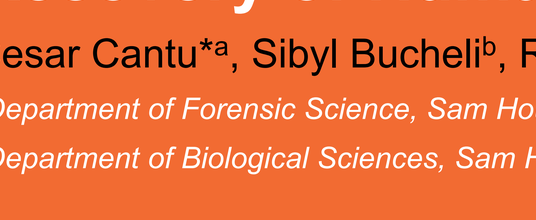

Figure 1 Representative Fly Larva with a Visible Crop. A) Intact Calliphoridae larva with brown crop prior to dissection. B) Larvae pinned and dissected, with the crop released, ready for DNA extractions.

MATERIALS AND METHODS

Sampling

Third-instar fly larvae were collected from three cadavers (**Table 1**) at the Southeast Texas Applied Forensic Sciences (STAFS) Facility and stored frozen until use.

Table 1 Sample Summary

Cadaver	Sample Type	Life Stage	Sample Size	Sample Identifiers
A	Crop	Third Instar	n=10	Crop
	Whole Fly Larvae	Third Instar	n=10	Whole
B	Whole Fly Larvae	Post-Feeding	n=5	Post
C	Crop-Burned Cadaver	Third Instar	n=5	Burned

Dissection

Fly larvae were washed with 20% bleach and rinsed with deionized water. Crop dissections were performed using a stereomicroscope and whole maggots were prepared by removing posterior spiracles and mouthhooks following Cantu et al. (9).

DNA Extraction

Human DNA was extracted from crops using DNeasy® Powersoil® Pro Kit (QIAGEN) manual purification following manufacturers protocol with 50 µL elution.

DNA Quantification

Human DNA was quantified using Quantifiler™ Trio DNA Quantification Kit (Applied Biosystems™) on a 7500 Real-Time PCR System (Applied Biosystems™).

RESULTS & DISCUSSION

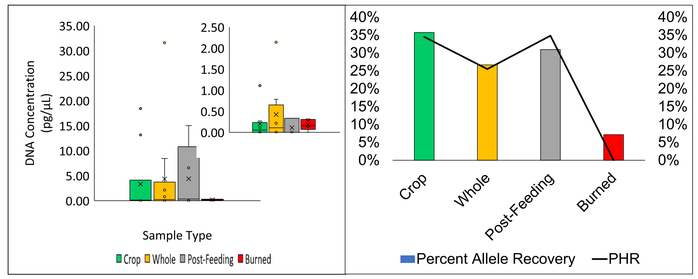

Figure 2 Human DNA Concentration and STR Typing. Left) DNA recovery is shown by sample type. DNA concentrations <2.5 pg/µL. Right) Average Percent Allele Recovery and PHR. Third instar crop samples had the highest allele recovery. Crops from fly larvae collected from Cadaver C, the burned cadaver, yielded the lowest percent allele recovery and PHRs.

Table 2 Sequence recovery and haplogroups.

Sample	24plex Allele Recovery*	Sequence Recovery+	EMPOP Frequency	Haplogroup
Crop-4	4%	100%	1.70E-04	U4b1+146+152
Crop-5	80%	100%	1.70E-04	U4b1+146+152
Crop-8	33%	100%	1.70E-04	U4b1+146+152
Crop-10	2%	85%	1.49E-03	U4
Whole-4	2%	100%	1.70E-04	U4b1+146+152
Whole-7	0%	100%	1.70E-04	U4b1+146+152
Post-1	7%	100%	3.57E-02	R0
Post-3	0%	100%	3.57E-02	R0
Post-4	0%	100%	3.58E-02	R0
Burned-1	7%	100%	5.34E-03	H5
Burned-3	16%	95%	5.55E-03	H5
Burned-4	4%	72%	7.41E-02	R0
Burned 5	11%	97%	5.49E-03	H5

*Green is ≥ 35% allele recovery, yellow is between 34% and 10%, orange is between 9% and 1% and red is 0% recovery.

+Green is 100% sequence recovery, yellow is between 99% and 90%, orange is between 80% and 89%, and red indicates < 79% recovery.

Table 3 Random Match Probabilities.

Sample	24plex RMP	MainstAY SE RMP*	Shared Alleles RMP*
Crop-1	7.662E+1	6.019E+1	6.019E+1
Crop-5	1.9614E+26	1.9007E+11	2.359E+10
Crop-8	2.6324E+6	-	-
Whole-3	5.8288E+25	2.8408E+26	2.8096E+21
Whole-9	1.8598E+6	1.6796E+9	1.5067E+7
Whole-10	4.1127E+14	9.2958E+14	2.2509E+11
Post-2	3.6115E+24	3.3693E+47	9.365E+34
Post-5	1.3678E+11	5.193E+0	5.193E+0
Burned-1	1.274E+1	7.7719E+4	1.355E+1
Burned-5	1.663E+2	2.737E+2	1.808E+2

*Green indicates RMPs higher than those calculated for Investigator® 24plex QS, yellow indicates an RMP that was lower. Red indicates 0% allele recovery using MainstAY and an RMP was not calculated.

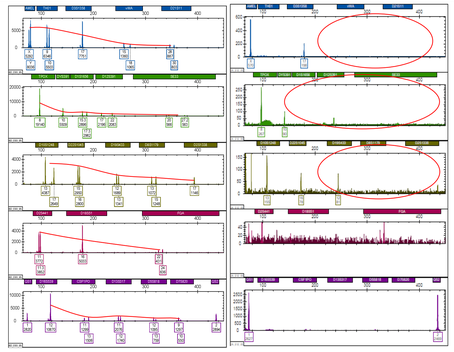

Figure 3 STR Profiles. Left) full profile from a crop extracted with Powersoil® Pro showing degradation, with the ski slope effect. Right) partial profile extracted with larger loci dropping out.

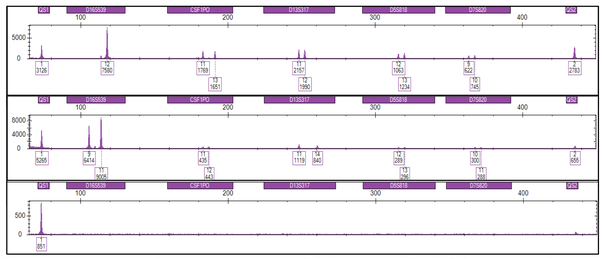

Figure 4 Inhibition Determined using QS Markers. Top) QS markers from a Cadaver A crop sample showing degradation and no inhibition. Middle) QS markers from a whole post-feeding fly larvae with inhibition detected, with QS2 <20% RFU of QS1. Bottom) QS markers from a whole post-feeding fly larvae showing inhibition with QS2 marker dropping out.

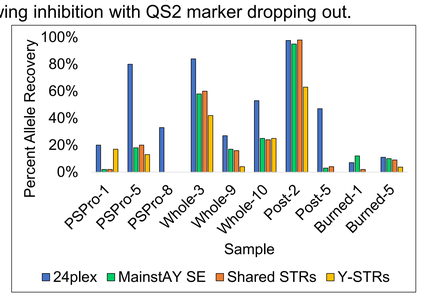

Figure 5 Percent Allele Recovery for MainstAY SE. Allele recovery of Investigator® 24plex and MainstAY SE is compared for select samples

- Powersoil® Pro can yield sufficient human DNA (**Fig. 2A**) for STR typing with a t-test showing no significant difference between crops and whole fly larvae.
- Full and partial profiles were recovered across the different sample types, with crops from Cadaver A (**Fig. 2B**) performing the best. STR profiles showed degradation, indicated by the typical ski slope effect and loci dropout (**Fig. 3**).
- Post-feeding fly larvae could remain inhibited, as indicated by the QS markers (**Fig. 4**). Post-feeding fly larvae have an increased insect fat body content, which is composed of lipids which can be PCR inhibitors.
- mtDNA can be used in missing person cases and to overcome degraded DNA. Thirteen samples (**Table 2**) with partial or no recoverable STR profiles were successfully sequenced and were concordant with reference haplotypes.
- Additional Y-STRs (**Fig. 5**) and higher RMPs (**Table 3**) were recovered for several samples using MainstAY SE. To the author's knowledge this was the first time NGS was used on human DNA recovered from fly larvae.
- DNA barcoding was successful (not shown), with six fly larvae identified as *Chrysomya rufifacies*, *Phormia regina*, and *Cochliomyia macellaria*. *P. regina* and *C. macellaria* had not previously been used for recovering human DNA. Additionally, there was no interference of the human DNA present. The species identified are of forensic importance as they can be used to estimate the post-mortem interval (PMI).

MATERIALS AND METHODS

STR Amplification

Amplification was performed using Investigator® 24plex QS Kit (QIAGEN). Capillary electrophoresis was performed on a 3500 Genetic Analyzer (Applied Biosystems™) and profiles were analyzed on ArmedXpert v.3.1.14 (NicheVision Forensics) with an analytical threshold of 50 RFU and stochastic threshold of 200 RFUs. ArmedXpert was used to calculate RMPs.

mtDNA Sequencing

Sequencing of HV1 and HV2 regions was performed on select samples using BigDye® Direct Cycle Sequencing Kit (Applied Biosystems™) and purified using BigDye® Xterminator™ (Applied Biosystems™). Capillary electrophoresis was performed on a 3500 Genetic Analyzer and analyzed on Geneious R7 (Biomatters). Sequences were compared to rCRS sequence and haplotypes were uploaded to EMPOP for haplogroup assignment and frequencies.

MainstAY SE

Ten extracts were selected for ForenSeq® MainstAY SE (QIAGEN) and prepared following manufacturer's recommendation. Sequencing was performed on a MiSeq FGx® with MiSeq FGx® Micro Reagent Kit (QIAGEN). Genotype calls were made using the Universal Analysis Software (UAS) v.2.5 and ArmedXpert was used to determine RMPs.

DNA Barcoding

Type-it ® Microsatellite PCR Kit (QIAGEN) was used to amplify COI from fly larvae using primers by Folmer et al. (10). Amplification products were purified using QIAquick® PCR (QIAGEN) Purification Kit and sequenced using BigDye™ Terminator 3.1 (Applied Biosystems™). Clean up was done using BigDye® Xterminator™ with capillary electrophoresis on a 3500 Genetic Analyzer. Sequence analysis was performed using Geneious R7 and sequences were queried against BLASTn to determine a species.

CONCLUSIONS

- Crop and whole fly larvae can be used to recover human DNA using Powersoil® Pro. Post-feeding fly larvae might have an increase in inhibitors, yet partial profiles were recovered from post-feeding fly larvae.
- mtDNA sequencing and MainstAY SE can be used to when STR amplification is unsuccessful, yielding additional probative information. Further investigation is necessary to determine which commercially available NGS chemistries would be the most beneficial.
- DNA barcoding can aid PMI estimations or potentially determine the geographical origin of fly larvae.
- Addressing existing knowledge gaps is necessary to demonstrate that fly larvae can yield robust and reliable results when analyzing human DNA. The evidentiary value of fly larvae can be enhanced by aiding entomological methods and recovering human DNA for various forensic genotyping analysis.

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