G25 Extracting Human DNA From the Crops of Maggots That Have Been Collected During Different Stages of Development and Preserved Using Different Methods

Jason G. Linville, BS, Department of Biology, University of Alabama at Birmingham, 2909 Highland Avenue, S Apartment 207, Birmingham, AL; Jeffrey D. Wells, PhD, Department of Justice Sciences, University of Alabama at Birmingham, 901 15th Street South, Birmingham, AL*

Upon completion of this presentation, participants will understand the effect of the maggot preservation method on the ability to extract human DNA from the maggot's crop and will understand how the size of a maggot and its crop affect the strategy for human DNA extraction from the maggot.

The type of corpse a maggot has been feeding on can be identified through DNA analysis of the maggot crop contents. The crop is a food storage organ located at the anterior end of the alimentary canal. Maggot dissection, followed by extraction of only the crop, is favored since it leaves the maggot's exterior available for identification purposes. Recently, Wells *et al*. identified several situations when maggot crop analysis would be useful in a forensic investigation (*J Forensic Sci* 46(3):685-687). DNA analysis could help investigators identify a missing victim if maggots are discovered at a suspected crime scene in the absence of a corpse. Maggot crop analysis also could provide a forensic entomologist with another way to associate a maggot with a victim. When making a postmortem interval (PMI) estimation, it is assumed the maggot's entire development took place on the victim. DNA analysis of the crop could reveal that the maggot had moved onto the victim from a different nearby food source. Maggot crop analysis also could be used to resolve a chain of custody dispute in which the origin of maggot evidence is in question.

The method of maggot preservation may affect the investigator's ability to successfully extract vertebrate DNA from the maggot's crop. The storage temperature and type of preservation fluid can alter the stability of human or other vertebrate DNA within the maggot crop. Also, the type of preservation fluid can change the physical characteristics of the maggot, which may inhibit the investigator's ability to dissect the maggot and remove the crop intact.

Another factor the investigator should consider during analysis is the maggot's stage of development. The size of the maggot and its crop may render different strategies for extracting vertebrate DNA from the maggot. Young maggots may be too small for dissection and crop removal. In older, post-feeding maggots, the maggot stops feeding and the crop contents are emptied into the remainder of the maggot gut. Alternative methods of analysis, such as extraction of the entire maggot, may provide better results for maggots that are too small for dissection, or for postfeeding maggots when the crop is no longer visible.

For the preservation study, maggots raised on human spleen were preserved using eight different preservation methods (70% ethanol, 95% ethanol, 4°C in 70% ethanol, 4°C, -70°C, room temperature, Kahle's solution and formaldehyde). Maggots were dissected after time periods of 2 weeks, 8 weeks and 6 months. Each maggot's crop was removed and extracted. Human DNA recovered from each crop was quantitated using Quantiblot® Human DNA Quantitation Kit (Applied Biosystems, Foster City, CA). Amplification of the human mitochondrial hypervariable regions (HVI, HVII) was attempted for all crop extractions. Amplification of STRs using Promega's (Madison, WI) Geneprint Powerplex 1.2 System was also attempted for all crop extractions. Successful HVI and HVII amplifications were sequenced using a PE-Biosystems (Foster City, CA) 310 genetic analyzer and BigDye Terminator® sequencing kit. Successful STR amplifications were analyzed using the 310 genetic analyzer.

Preliminary results suggest that the preservation method does affect the ease of dissection and the quantity of DNA recovered. For example, in maggots preserved in 95% ethanol at room temperature, the dehydrated crop became attached to other internal organs, often resulting in a broken crop during dissection. In maggots preserved in formaldehyde, although the crop was easily removed, quantitation results suggest a reduced amount of DNA had been extracted, in some cases preventing the amplification of HVI and HVII regions. Additional preservation results will be discussed.

For the development study, maggots raised on human spleen were removed and preserved at half-day intervals until the maggots began to pupate. The collected maggots were dissected and, if possible, the crops were removed and extracted. In maggots that were too small for crop removal, the entire maggot was extracted. In older, post-feeding maggots that no longer contained a visible crop, the intestines were extracted. DNA sequencing and STR analyses were performed as described above.

Preliminary results suggest that in maggots too small for dissection, extraction of the entire maggot did allow for the recovery of human DNA. However, in older, post-feeding maggots, extraction of the intestines did not result in the recovery of human DNA. Additional results will be discussed.

The results demonstrate that the chosen preservation method does have an effect on the ability to dissect a maggot and on the quantity of DNA extracted from the crop. Also, recovery of human DNA is possible in maggots that are too young for crop extraction, but is not likely in older post-feeding maggots with empty crops.

Forensic Entomology, Maggot Crop, Mitochondrial DNA

 ** Presenting Author*