## D2 Species Composition of the Maggot Mass

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After attending this presentation, attendees will learn how molecular identification techniques were used to validate the hypothesis that the species composition of the maggot mass is comprised of multiple species.

This presentation will impact the forensic science community by determining the species composition of the maggot mass to be a single or multiple species complex. A maggot mass that is composed of multiple species will bear significant impact on insect development studies since the presence of one species can slow or accelerate the developmental rate of another species. These developmental rates will have a direct effect on the postmortem interval estimation.

During initial human decomposition, it has been observed that several adult female fly species visit the body. Even closely related carrion species can differ in their growth rates, diapause response, and/or ecological habits. Therefore, accurate identification of an insect specimen is crucial. Immature stages of many forensically significant species are notoriously difficult to identify in early developmental stages (instars) and lack defining anatomical characteristics. Identifying each maggot according to distinguishing morphological characteristics can be a time-consuming task. In addition, the process can suffer from human error if performed by an untrained forensic entomologist. For these reasons, DNA-based methods of identification should be used.

Currently, the maggot mass is assumed to be of multiple species composition, but this idea has not been validated. Therefore, it is unknown if adult female flies of different species will lay their eggs in the same location on a corpse as other adult female flies. This study will test the hypothesis that a maggot mass is composed of several different species of larval flies. This project will employ the use of automated DNA sequence analysis standards and phylogenetic methods to compare "unknown" maggots to "known" adult flies. Co-Oxydase enzyme I (COI) and Co-Oxydase enzyme II (COII) gene sequences will be amplified and sequenced. COI and COII are unique markers that are highly conserved because they code for respiratory processes (electron transport); and are species-specific genes. The combination of slowly evolving regions coupled with wobble regions makes these genes a perfect marker to use in molecular species identification techniques.

Adult flies and maggots will be collected from three different cadavers during June, July, and August of 2011. Flies will be actively collected with nets for five consecutive days once the human body has been placed. The adult flies will be stored in vials of 95% alcohol until they are identified by species via use of published dichotomous keys, a reference collection, and an Olympus SZX16 stereomicroscope equipped with a DP72 color digital camera. Adults will be stored and saved to serve as positive controls for the maggot DNA sequencing. After one week, first and second instar maggots from the initial maggot mass will be collected and stored in 95% ethanol at 80°C. Fifty maggots from each body will be randomly selected and identified by molecular techniques that have been proposed and validated by forensic entomologist Jeffrey D. Wells[1] and the Bucheli Lab (in prep).

Gut content from frozen maggots will be cut and removed. The remaining maggot muscle tissue will be blended in a tissue homogenizer and DNA will be extracted according to a Chelex extraction protocol. Purification of the extraction will be performed with phenol chloroform protocol. DNA will be quantified by ultraviolet (UV) spectrometry. Polymerase Chain Reaction (PCR) will amplify samples on the GeneAmp PCR System 9700 thermal cycler. Promega Hot Start polymerase master mix will be used in conjunction with primers published by Wells[1] to generate the PCR products. The resulting amplicons will be cleaned using a QIAquick PCR purification kit from QIAGEN. An agarose gel check will be performed to ensure DNA has been amplified prior to sequencing. The sequence will be detected on the Applied Biosystems 3500 Genetic Analyzer with BigDye Terminator version 3.1. The Geneious Pro cross-platform bioinformatics software suite will be used to analyze the resulting sequences. Phylogenetic analysis will be used to perform identification of the maggots sampled from the initial maggot mass. Parsimony analysis will be used to construct a phylogeny where the unknown maggot species and known maggot species will be used. The sister

 

relationship of unknowns to knowns will be used for identification criterion. Bootstrap and jackknife statistics will be calculated to assess clade support and the confidence of the sisters relationship.

**Reference:**

1. Wells JD, Sperling FAH. DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). Forensic Science International, 2001. 120:110-115.

**Forensic Entomology, DNA, Postmortem Interval**

 