D49 Mitochondrial DNA-Based Identification of Family Calliphoridae and Sarcophagidae

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The goal of this presentation is to present to the forensic community the implementation of mitochondrial DNA methodologies and sequence data from population samples of a calliphorid and sarcophagid species in Hawaii.

This presentation will impact the forensic community and/or humanity by providing the sequence data from sarcophagids and calliphorids from Hawaii, which will be entered into GenBank so that the forensic community may have population samples from this location to aid in quick identification of these species for estimation of postmortem interval.

Forensically important insects collected on a decomposing body offer a unique opportunity to estimate time of death (or postmortem interval, PMI) if the species can be positively identified. Many adult carrion-flies are easily distinguishable, but the larvae are not. The most common flies to inhabit a human corpse in Hawaii are blowflies from the family Calliphoridae and fleshflies from the family Sarcophagidae. Sarcophagid flies have many characteristics that make them ideal forensic indicators. However, their utility is limited because it is difficult or impossible to determine the species of a sarcophagid larva (Wells Pape and Sperling 2001). The same holds true for calliphorid species in different developmental life stages. Identification of the immature blowfly larvae is more difficult and sometimes impossible (Schroeder Klotzback 2002).

The rationale for this study follows the work of Wells and Sperling et al (2001) by providing an alternative method to using morphology in identifying indistinguishable larvae by use of mitochondrial DNA (mtDNA) sequence data and phylogenetic analyses. The collection of his work throughout the years and the work of other scientists has produced a useful database providing sequences to different arthropod species. The database, however, is limited to species common on the mainland and continental regions. Since the Hawaiian Islands are the most isolated archipelago in the world, with regular species likely introduced from Asia, Australia, the U.S. mainland and other Pacific Islands, the array of sarcophagids and calliphorids in Hawaii is slightly different than in any of these individual locations. As Hawaii blowflies and fleshflies represent populations different from their ancestral (Asian, Australian, etc.) ones, sequences for a given species in Hawaii may likely differ from those reported thus far. Adding sequence data from such flies found in Hawaii, will be useful to compare these with already published sequences, especially if the sequences for a given species differ from previously reported ones. Therefore, the study is assuming that mtDNA regions will be similar but not identical from species in different locations and that isolation of mtDNA from larvae will be relatively straightforward.

In this pilot study, an organic extraction of mtDNA was made with single flies and single larva of Chrysomya megacephala and Sarcophaga ruficornis. Specific fragments of the cytochrome oxidase subunit one (COI) region of the mitochondrial DNA were amplified using polymerase chain reaction (PCR). Locations of primers used in this study were taken from Wells and Sperling et al 1999 – CI-J-2183 and CI-N-2659. Amplified sequences were obtained and sequenced at the Biotechnology CORE facility at the University of Hawaii Manoa Campus using two Applied Biosystems 377XL DNA Sequencers. Analysis of sequences were compared to Wells and other published sequences accessible online using the BLAST search engine of the National Center for Biotechnology Information. For each pair of species example, the amplified fragment was approximately 523 base pairs long in C. megacephala and approximately 498 base pairs long in S. ruficornis. Sequences are currently being analyzed, and a full report will be presented in the poster. Initial study indicates between 53 and 58 nucleotide changes between C. megacephala and S. ruficornis in this region. Sequences from the Hawaii specimen of C. megacephala were very similar but not identical to previously reported Chrysomya megacephala from Australia or South Africa, having 7 nucleotide differences. Preliminary sequence analysis and searches in GenBank with S. ruficornis indicated the closest relatives were Chrysomya norrisi and Lucilia adisoemartoi having 41 nucleotide differences each, from both species. Results obtained aid in the quick identification of sarcosaprophagous arthropods in estimation of postmortem interval (PMI). Mitochondrial DNA is a successful and valuable tool in the application of forensic science. This research was supported by USDE grant #P217A030070.

Mitochondrial DNA, Calliphorids, Sarcophagids

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