B14 Mitochondrial DNA-Based Identification of Forensically Important Sarcophagidae and Calliphoridae (Diptera) in Hawaii

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After attending this presentation, attendees will learn about how to identify different species with mitochondrial DNA sequencing, and how important this is to forensic entomologist in order to determining the postmortem interval correctly.

This presentation will impact the forensic community and/or humanity by giving them samples of Sarcophagidae and Calliphoridae species from Hawaii. The mitochondrial DNA sequences will help law enforcement in determining postmortem interval.

The poster will present partial mitochondrial DNA sequence analysis from DNA isolated from larvae and adult flies of the Sarcophagidae and Calliphoridae families in Hawaii. The mitochondrial DNA sequences from Hawaii are similar but not identical to flies from other locations around the world.

Organic extraction such as CTAB, Pure Gene, and other methods were used to isolate amplifiable mitochondrial DNA, Centricon YM-100 filters were used to further purify the template. The *Sarcophaga ruficornis* DNA extraction sample contained a concentration of .5151g/1l times 501l= 25.751g and the *Chrysomya megacephala* DNA extraction sample contained a concentration of 1.311g/1l times 1001l= 1311g. The samples were diluted down to a concentration of 10ng/1l, and between 30 and 70 ng templates were used in each PCR reaction. The pair of primers used were TY-J-1460 and CI-N-1840. The Annealing temperature used in the thermocycler was at a set temperature of 41ºC for 30 complete cycles. Amplification product was verified by a 1% agarose gel electrophoresis containing Ethidiumbromide stain, and yields estimated for subsequent sequencing with the DNA molecular weight marker XIV (100-1500bp). Amplification was successful in all of the larvae and adult fly samples. The samples were sent to the Biotechnology Core Facility at the University of Hawaii in Manoa for sequencing in an Applied Biosystems 377XL DNA Sequencer. The sequences analysis was based on a region between 400 base pairs of the gene for cytochrome oxidase subunit one (CO1). The sequences for the *Sarcophaga ruficornis* and the *Chrysomya megacephala* were then compared in an online BLAST search engine of the National Center for Biotechnology Information's Genbank. The results from the sequence comparison for the *Chrysomya megacephala* in Hawai'i were similar but not identical to the *Chrysomya megacephala* from between Lae and Bulolo, Papua New Guinea (Wells and Sperling, 2000) (Calliphoridae, GenBank accession AF295551). The results from the sequence comparison for the *Sarcophaga ruficornis* in Hawaii did not result in a close sequence similarity with the *Sarcophaga ruficornis* Genbank entry (AF259511) but instead it closely resembled a *Sarcophaga africa* sequence from Berkeley, California, a related species (Wells, Pape, and Sperling, 2000) (Sarcophagidae, GenBank accession AF259508). The *Sarcophaga ruficornis* sequence was also found to be similar to *Sarcophaga crassipalpis* from Berkeley, California (Wells, Pape, and Sperling 2000) (Sarcophagidae, GenBank accession AF259510).

The conclusions of this research resulted in isolation of amplifiable DNA for larvae and adult flies in Hawaii. Mitochondrial DNA sequences from these species in Hawai'i are similar but not identical to the fly species from other regions around the world. This research also shows evolutionary difference between the two specific species in different regions. Being able to identify the Calliphoridae and Sarcophagidae families more easily will help the forensic community in determining the postmortem interval in Hawaii. This work was supported by USDE grant number P217A030070.

Mitochondrial DNA, Sarcophagidae or Calliphoridae, Species Identification

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