

FSCI 101: Introduction to Forensic Science
Lab: Forensic Entomology Part II; Bioinformatics Tools

Purpose: The purpose of this laboratory is to introduce students to the use of modern computational resources in the analysis of DNA evidence. We will utilize bioinformatics tools to address questions on forensic entomology that cannot be solved using traditional morphological analyses.

Overview of Arthropods in Forensic Science

The study of insects as they relate to questions in an ongoing criminal investigation has become an increasingly important sub-discipline in forensic science. Forensic entomology focuses on the interpretation of arthropod evidence as it relates to a crime, usually violent in nature. Entomological evidence is well suited to help an investigator address two important questions: (A) estimation of the total time a decedent has been dead (postmortem interval or PMI) and (B) where the crime occurred. Recent advances in forensic entomology have shown the arthropod evidence can provide information about the surface scatter of buried bodies, toxicology of deceased victims and alteration of bloodstain evidence at a crime scene.

The postmortem interval can be accurately determined in cases where arthropods are present at the crime scene due to well-known, temperature dependent lifecycles of insects as well as a predictable progression of different insect species on the decedent as part of the natural decomposition cycle of organic matter.

The proper identification of the insects found at the scene of the crime is the first and most important step in any forensic entomological investigation. Only by a correct identification of insects present can their developmental stage be correctly identified and then linked to the temperature at the site of the crime. Many times, species identification can be made by a simple morphological examination. Table 1 lists some of the most important arthropods in an investigation.

Insect Common Name	Important Designations
Blue Blow Flies	<i>Calliphora vicina, Calliphora vomitoria</i>
Oriental Latrine Fly, Hairy Maggot Blow Fly	<i>Chrysomya megacephala, Chrysomya rufifacies</i>
Secondary Screwworm Fly	<i>Cochliomya macellaria</i>
Flesh Fly, Red-tailed Flesh Fly	<i>Sarcophaga bullata, Sarcophaga haemorrhodalis</i>
Common Dung Fly	<i>Scatophaga stercoraria</i>
Carrion beetles	<i>Family Silphidae</i>

Table 1: Important Insects in Forensic Investigation

Traditionally, the identification of insects has been accomplished through morphological analysis of important features of the larvae, pupa or adult insect.

Traditional Identification Tools for Arthropods

There are several common points of comparison for insect identification. When trained forensic entomological experts are unavailable, the use of simple keys can make a rough assignment of species classification available to a generally trained scientist. Important morphological features in the identification of insects include: size/shape of eyes; shape of antennae structures; presence & location of spiracles; size & color of thorax; size & color of abdomen.

Use of DNA for Identification of Arthropods

With increasing availability of DNA methods in the forensic investigation, there has been an emerging interest in using DNA techniques to assign species classification to insects recovered from crime scenes. Flies in particular are well-suited to DNA analyses since large numbers of fly sequences are maintained in the GenBank.

The use of mitochondrial DNA (mtDNA) is of increasing importance in forensic science. In human subjects, applications in forensic science are limited to maternity testing and possible identification using HV1 & HV2 (Hyper Variable) regions of human mitochondria. Since mtDNA is present in high copy number in human cells, current interest is focused on using mtDNA evidence when nuclear DNA techniques cannot be applied, usually due to the age and/or deterioration of the sample.

mtDNA analysis of insects uses sequence information in the Cytochrome Oxidase Region (either I, II or both). Sequencing COI or COII will allow investigators to identify the insect by comparing it to known sequences. The bioinformatics tool that will allow us to accomplish this goal is known as a Basic Local Alignment Search Tool for Nucleotides (BLASTN) search. A BLASTN search will provide a match (if available) from a laboratory determined sequence to a sequence maintained in GenBank or another accessible repository of data. It will also return the closest possible matches to the sequence in question.

Phylogeny

Since the analysis of insect mtDNA is relatively limited, there is an unknown amount of halotype diversity even between closely related species. When an exact DNA match cannot be retrieved by a BLAST search, a phylogenetic analysis can reveal the closest likely relatives to the DNA sample in question. The bioinformatics tool that will allow us to do this is called CLUSTAL. This

program facilitates multiple sequence alignment and can generate phylogenetic trees.

Procedure

For this laboratory, we will utilize two bioinformatics programs to answer questions of importance in a hypothetical investigation. Once we have determined the species, we will carry out a standard PMI calculation using meteorological data and Accumulated Degree Days (ADD) (see *Introduction to Forensic Entomology Lab* for more details).

Case Study: The body of an unknown female was found dumped along a secondary road outside of town on August 21, 2006. A local hiker walking along the road noted an unusual odor and discovered the decedent in an industrial garbage bag. The body was wrapped in a sheet. The bag had a small hole at the bottom which allowed for insect access to the corpse with primarily larval specimens present. There were no signs of other scavengers or predation upon the deceased. Samples were collected from the body before its removal and meteorological data was tabulated.

The crime lab was able to identify the larval samples of being in the 3rd Instar of development however, a specific species identification could not be established. The insect samples were sent to a laboratory for mitochondrial DNA analysis. The laboratory sequenced the Cytochrome Oxidase Subunit I (COI) for all samples. The data returned indicated the presence of two distinct sets of insects present at the scene.

- 1). Access the workstation using your assigned login. On the desktop, you should locate the two sequence files labeled *Fly Sample #1* and *Fly Sample #2*.
- 2). Open a *Safari* webpage and access the San Diego Super-Computing Center at: <http://workbench.sdsc.edu>
- 3). Login using the user name and password given to you by the instructor.
- 4). First, we will attempt to find an exact match for each of the sequences. To do this, we will need to load the sequence files into the Workbench.
- 5). Open the *Fly Sample #1* file on your desktop.

- 6). Next, click on the *Nucleic Tools* button on the Workbench interface. From here, select the *Add New Sequence* option from the scroll-box and click *Run*.
- 7). Label the sequence with an appropriate title (i.e. "Unknown Fly Sequence #1") and copy the contents of the Word file into the Sequence box. Once this is complete, click *Save*. Your sequence will be uploaded and you should return to the *Nucleic Tools* page.
- 8). We will now attempt to match the unknown laboratory sequence to a sequence available in one of the main databases. Click on your sample. Then select *BLASTN* from the list of options and click *Run*. The next screen will allow us to select that appropriate database to scan. Select *GenBank Invertebrate Sequences*. Leave the other options the same and then click *Submit*.
- 9). A large list of possible matches will be returned, sorted by score. What is the highest scoring match? Is it an exact match? What % of the identities matched successfully? Are there any other close matches?
- 10). Repeat the above steps for Fly Sequence #2.
- 11). Note with Fly Sequence #2, there is no exact match. We can use another bioinformatics tool to generate a phylogenetic tree to examine relationships between the closest possible matches.
- 12). From the *BLASTN* results screen, check the top 10 scoring matches and click the *Import Sequences* button. Once back on the *Nucleic Tools* page, check all ten of the imported sequences. Then select the *CLUSTALW* program from the scroll-box and click run. Leave the options screen unchanged and click *Run*. This will align the multiple sequences. The data output shows conserved residues in blue. Click the *Import Alignment* button.
- 13). Check the *CLUSTALW* alignment and select the *DRAWGRAM* program from the scroll-box and click *Run*. On the following screen, change "*Exclude positions with multiple gaps*" to *Yes* and "*Correct for multiple substitutions*" to *Yes*. Then click *Submit*.
- 14). The program generates a rooted phylogenetic tree. Individual sequences are labeled by GenBank Ascension number.
- 15). Now that you have probable identifications of the two insects recovered from the decedent, use the meteorological data and appropriate developmental data in the Appendix to estimate the PMI.

Laboratory Report:

The laboratory report for this lab will be done in the standard short memo format used previously. Refer to “*Writing a Technical Memo*” handout from Week 1. You should highlight the following areas in your memo:

- ❖ What were the results of the BLAST searches on the two sequences?
- ❖ What information did the CLUSTAL run provide? What conclusions did you draw from this data?
- ❖ Summarize your calculations and conclusions for a probable PMI. Be sure to briefly describe the methodology you used to arrive at this figure. Actual calculations, graphs & figures should be imbedded in the memo if they are critical otherwise included in the Appendix.
- ❖ Did the fact that the body was wrapped in a bag influence your PMI calculations? If so, explain how.
- ❖ Comment on your impressions of the application of mtDNA sequencing of arthropods in investigations. When should this technique be considered by an investigating laboratory?

References

Byrd, J. H; Castner, J. L. eds. *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. CRC Press: New York, 2001.

Wells, J. D.; Sperling, F. H. *J. Med. Entomol.* **1999**, *36(3)*, 222-226.

Wells, J. D. *et al. J. Fors. Sci.* **2001**, *46(3)*, 685-687.

Sperling, F. A.; Anderson, G. A.; Hickey, D. A. *J. Fors. Sci.* **1994**, *39(2)*, 418-427.

Wells, J. D.; Pape, T. ; Sperling, F. A. *J. Fors. Sci.* **2001**, *46(5)*, 1098-1102.

APPENDIX

Meteorological Data for Scene

Date	Max °F	Min °F
8/21/2006	90	70
8/20/2006	91	69
8/19/2006	92	70
8/18/2006	91	68
8/17/2006	94	68
8/16/2006	90	71
8/15/2006	92	71
8/14/2006	92	71
8/13/2006	88	71
8/12/2006	91	73

Developmental Data for Cochliomyia macellaria

Stage	At 15.6 +/- 2 °C Hours to Reach Stage	At 21.1 +/- 2 °C Hours to Reach Stage	At 27.6 +/- 2 °C Hours to Reach Stage
1 st Instar	32	12	18
2 nd Instar	70	30	32
3 rd Instar	122	72	56
Pupa	276	172	112
Adult	588	297	177

Developmental Data for Sacrophaga bullata

Stage	At 15.6 +/- 2 °C Hours to Reach Stage	At 21.1 +/- 2 °C Hours to Reach Stage	At 27.6 +/- 2 °C Hours to Reach Stage
1 st Instar	*	*	*
2 nd Instar	14	12	6
3 rd Instar	72	48	26
Pupa	262	160	110
Adult	802	504	252

Developmental Data for Chrysomya rufifacies

Stage	At 15.6 +/- 2 °C Hours to Reach Stage	At 21.1 +/- 2 °C Hours to Reach Stage	At 27.6 +/- 2 °C Hours to Reach Stage

1 st Instar	46	26	18
2 nd Instar	54	30	24
3 rd Instar	154	92	78
Pupa	298	128	82
Adult	598	296	216

Developmental Data for Calliphora vomitoria

Stage	At 15.6 +/- 2 °C Hours to Reach Stage	At 21.1 +/- 2 °C Hours to Reach Stage	At 27.6 +/- 2 °C Hours to Reach Stage
1 st Instar			
2 nd Instar			
3 rd Instar			
Pupa			
Adult			

Developmental Data for Sarcophaga haemorrhoidalis

Stage	At 15.6 +/- 2 °C Hours to Reach Stage	At 21.1 +/- 2 °C Hours to Reach Stage	At 27.6 +/- 2 °C Hours to Reach Stage
1 st Instar			
2 nd Instar			
3 rd Instar			
Pupa			
Adult			