H91 Transcriptional Markers of Sex Determination for Forensic Entomology

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Learning Overview: After attending this presentation, attendees will understand how genetic markers can be used to predict the sex of blow fly species of forensic importance, which can aide in determining more accurate Postmortem Interval (PMI) estimates.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by identifying genes that can be used for a transcriptional approach to sex identification in blow fly species of forensic importance: *Lucilia sericata* (Diptera: Calliphoridae) (Meigen), *Cochliomyia macellaria* (Diptera: Calliphoridae) (Fabricius), and *Chrysomya rufifacies* (Diptera: Calliphoridae) (Macquart).

Indication of sex when identifying insects from human remains is important for death investigations in forensic science. The age and sex of insects is integral for PMI estimates when assumptions for certain conditions can be met.[1] In forensic entomology, species-specific data is gathered for development rate under different temperature conditions.[2] This data can then be applied to case samples and a minimum PMI can be determined utilizing the known data sets.[1] Within these estimates, there can be error in not considering differences in development times between males and females of blow fly species. Currently, only adult specimens can be identified for sex by sight identification, while immature forms have no standardized method for sex identification.[3] This can lead to inaccuracies in PMI estimates as this difference in development time is not being considered in the estimates. The objective of this research is to create an assay that will address the issue of sex identification in immature blow fly forms that can be applied to forensic casework, yielding more accurate PMI estimates.

Three species, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae), and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) were selected for this study. These species are all prominent within the state of Texas and have varying importance in widespread fields of entomology, such as veterinary entomology for myiasis of sheep, use in medical entomology for wound debridement therapy, and importance in forensic entomology, as they are all commonly found at remains recovery scenes.[3] Developmental differences between males and females within these species can be attributed to sexual dimorphism.[4,5] Within the sex determination pathway, differential splicing plays an important role.[4,5] It occurs during gene expression and will give rise to sex-specific phenotypes.[4,5] For this project, two genes of importance within the sex determination pathway—*transformer* (*tra*) and *doublesex* (*dsx*)—were targeted for analyses.[4,5] For the analyses, whole RNA was extracted and converted to cDNA. Then gradient Polymerase Chain Reaction (PCR) was used to determine the best annealing temperature for the selected primer sets and splicing products were visualized with gel visualization. Products and negative controls were confirmed with quantitative PCR (qPCR).

Currently, three distinct assays have been created that can identify the sex of immature forms within the selected blow fly species. Using accepted methodology within the field, these assays were created to target the *tra* sequence in *C. macellaria* and *L. sericata* and the *dsx* sequence in *C. rufifacies*. In addition to the assays created, an identification and error rate for this assay were determined to align with the need for known error rates within the field of forensic science. The assays will help advance forensic entomology by allowing for more accurate postmortem interval estimates in death investigations.

The application of this work can be seen in other projects as well. Currently, there is known gene expression data for *L. sericata*, as well as in progress gene expression data for *C. macellaria* and *C. rufifacies*.[6] With the help of this assay, we can determine whether gene expression has bias in the sexes and the amount of expression each sex may see. This assay can also be useful outside of forensic use in veterinary and ecological applications, such as for sex-specific biological control and better understanding sex-specific traits that may arise in species.[7] With this assay, not only will the field of forensic entomology benefit, but many other fields of entomology as well.[7-9]

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