H91 Validation of Punitive Markers of Age in Immature *Lucilia sericata* (Meigen) (Diptera: Calliphoridae)

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After attending this presentation, attendees will have a greater appreciation and understanding of the role that genetic markers can play in predicting insect age, which can determine the Postmortem Interval (PMI) when certain assumptions are satisfied.[1]

This presentation will impact the forensic science community by identifying genes that are uniquely expressed in *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) at the third instar and pupal stages that can be used to improve the precision of entomological PMI estimates.

L. sericata is a cosmopolitan blow fly of forensic, medical, and veterinary importance.[2] In regard to their forensic application, *L. sericata* collected from remains can be informative as to estimating the PMI. Historically, characteristics such as body size or immature morphological feature have been used to estimate fly age, but are not useful for determining how long an individual has been in that stage.[3] This can be problematic in the stages that persist for relatively long periods of time, such as the third instar and pupal stages. Research has shown that there are temporal patterns of gene expression throughout the immature development of insects.[4,5] Dipteran gene expression, in particular, has been of recent interest in forensic entomology.[3,6-8] Such biological information can be used to break lengthier stages into smaller temporal pieces and improve the precision of entomology-based PMI estimates. While this is promising information, there is a need for validation of these markers. This study identified 45 promising loci for validation.

Immature, colony-reared *L. sericata* of a known age were sampled and flash frozen every 12 hours throughout their development. Sex of individuals sampled was determined by *tra* Polymerase Chain Reaction (PCR) assay. RNA was extracted following a standard trizol prep that is similar to a phenol-chloroform method followed by the addition of SUPERase•In™ to prevent RNA degradation. Extracts were obtained from a total of 96 larval and pupal specimens of eight different ages (12 samples per age, from four distinct biological replicates). The RNA was then purified using an on-column DNase treatment, then converted to complimentary DNA (cDNA) for quantitative PCR (qPCR). A total of 45 markers were chosen based on a previous *L. sericata* genomic screen.[9] Genes from different clusters of co-expressed genes were selected to maximize the power obtained from adding genes to the analysis. These genes were known to be expressed at relatively high levels in one or more of the time points studied and had evidence of biological support from other species such as *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) based on comparisons to expression reported in FlyBase.[10] Three housekeeping genes, *rp49*[6], a tubulin gene[6], and *Ef2b*[9] were used to measure and compare levels of expression. Primers were designed prior to qPCR so they could be tested to determine the appropriate concentration needed for the validated markers.

Uncertainty surrounding PMI estimates based on insect age has been a concern for the forensic entomology community.[11,12] Gene expression is one way to improve imprecision in age estimates. This project is focused on a subset of genes identified as promising candidates and evaluated differences in the sexes. Ultimately, the best markers of fly age will be validated across numerous genotypes, environments, and research groups. Outside

of their forensic application, evaluating *L. sericata* gene expression could lead to better understandings of their evolutionary and developmental histories. Additionally, future research comparing lab-reared to wild-type gene expression profiles can also aid studies in development and behavior.

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