G74 The Validation of Gene Expression as a Means of Predicting the Age of the Blow Fly *Lucilia sericata*

Aaron M. Tarone, BS, Department of Zoology, Michigan State University, 203 Natural Sciences, East Lansing, MI 48824; and David R. Foran, PhD, Forensic Science Program, Michigan State University, 560 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand the use of gene expression in determining the age of forensically useful blow flies.

This presentation will impact the forensic community and/or humanity by helping the forensic community understand how to use gene expression and statistical analyses to better address the requirements of *Daubert* as it pertains to forensic entomology.

Forensic entomology is an established field, with a relatively good track record of predicting a postmortem interval (PMI) through an understanding of blow fly development. However, in the context of *Daubert*, all of the forensic sciences will be expected to provide techniques that have been adequately tested, have laboratory standard operating procedures (SOPs), have undergone publication in the peer-reviewed literature, are accepted by the relevant scientific community, and have known potential error rates. Though forensic entomology can meet most of these criteria, the field will likely be required to establish SOPs and provide information as to error rates and the precision of its methods.

In addition to not yet fully meeting *Daubert* requirements, blow fly based PMI estimates suffer from increasing error as the animal develops (Wells and Lamotte 1995). This stems from the fact that the durations of subsequent developmental stages increase, and the body size traits used by entomologists (larval length or weight) that help refine age estimates in the feeding stages become far less useful in the later stages of immature development. Thus, other characteristics that predictably change during these stages are potential sources of information that could be valuable to investigators attempting to age flies. Gene expression profiles are known to vary significantly throughout the development of all animals, and have been studied extensively in the dipteran *Drosophila melanogaster* (e.g., Arbeitman et al. 2002). To understand how gene expression might be utilized for more accurately aging a forensically useful blow fly, a gene expression profile data set for nine developmentally variable genes was created for the immature life cycle of ~700 individual *Lucilia sericata*. Three regional strains of flies were grown under controlled laboratory conditions and regularly sampled for both size and gene expression levels, creating a high-resolution developmental profile of these cohorts.

Following this, genetic profiles were used in conjunction with developmental stage and body size data to estimate the age of individual blow flies. However, body size and gene expression levels are not easily explained by a simple mathematical function (they are non-parametric) and statistical endeavors that can help investigators (and triers of fact) understand such multivariate and non-linear data must be employed to make predictions of age. To this end, statistical models of developmental age in terms of genetic and phenotypic profiles have been produced. The two most promising forms are principle component analysis and generalized additive models. The former has been used in other forensic sciences to make predictions of class evidence and may be useful with entomological data. With generalized additive models, it is possible to define the percent of development explained by the data, and to deduce the usefulness of different models (and variables within models) through a comparison of generalized cross validation (GCV) scores (Wood 2006). Currently, predictions of age incorporating standard data and gene expression can describe up to 97% of the variation in development, which is an increase of ~10% compared to using developmental stage and body size alone.

However, no mathematical model is useful based solely on its theoretical ability to predict age, thus the models need to be validated on different data sets. To accomplish this, a blind study was conducted with flies grown under both experimental (in the laboratory using the same conditions as the original experiment) and natural (out of doors) environments. Gene expression and body size profiles of the juvenile flies collected were used to predict the age of the individuals employing the models generated from the aforementioned data set. The validation of the age models and their importance in helping the field of forensic entomology increase precision and meet the requirements of *Daubert* will be discussed.

Forensic Entomology, Postmortem Interval, Generalized Additive Model

** Presenting Author*