G160 Analysis of the *De Novo* Transcriptome of Immature *Chrysomya Rufifacies* (Diptera: *Calliphoridae*) to Investigate Sexually Dimorphic Patterns of Gene Expression and Their Role in M-PMI Estimates With a Forensically Important Fly

Meaghan L. Pimsler, BS, Sing-Hoi Sze, PhD, Jeffery K. Tomberlin, PhD, and Aaron Tarone, PhD, Texas A&M Univ, College Station, TX 77843*

After attending this presentation, attendees will gain a better understanding of sexually dimorphic patterns of gene expression throughout the immature development of a blow fly of international forensic relevance. Furthermore, members will acquire insight into the application of these data to the development of sex-specific growth development curves and molecular methods for estimating ages of immature blow flies.

This presentation will impact the forensic science community by adding to the body of molecular data available for forensically relevant species of flies, which will help in refining time of colonization estimations using insect evidence in immature stages. Currently, the pupal stage is one of the most difficult stages in which to make an estimate of age, due to its externally quiescent nature. While predictable developmental changes are occurring in pupae, a very detailed understanding of fly development is required to make even qualitative refinements of pupal age with morphological data alone. The application of these genomic tools will enable researchers to use gene expression profiles to estimate age more accurately and reliably.

Previous work has identified temporal patterns of gene expression throughout immature development in arthropods,[1] such as Diptera,[2-4] and there is a wealth of research demonstrating sex-specific patterns of gene expression.[5-9] Other work has suggested that, in addition to differences in temporal patterns of gene expression, the sexes of the blow fly *Lucilia sericata* may also develop at different rates (Picard, personal communication). As a result of this, it may be possible to investigate molecular mechanisms governing this plasticity and develop predictive models that incorporate sex, gene expression, and accumulated degree hour data. Though there is a well-annotated and manually curated database, "FlyBase,"[10] of gene expression profiles throughout development in *Drosophila* (Diptera: *Drosophilidae*), a greater breadth of temporal gene expression profiles across Diptera will help researchers to better understand the evolution of developmental pathways and patterns of conservation. Further development of sex-specific differences in temporal gene expression patterns should also prove informative. *Chrysomya rufifacies* (Diptera: *Calliphoridae*) (Macquart) is an especially tractable model organism for these kinds of questions as it exhibits monogenic sex determination with single-sex offspring clutches and homomorphic sex chromosomes.[11-13]

In this experiment, offspring from an isolated female *Ch. rufifacies* were collected and flash frozen. The sex of each larva sampled was determined once the remainder of the cohort had eclosed. For each sex (male and female) and time-point, samples from six separate cohorts were collected. RNA was extracted, sequenced with Illumina HiSeq, and assembled with custom software similar to ASplice.[14] Analysis of the transcriptomes yielded putative markers of the stages of interest. The most promising markers were verified by designing primers based on predicted transcripts and testing them via qPCR.

Many of the genes with homology to those found in other organisms exhibited differential expression profiles across immature stages in accordance with patterns identified in *Drosophila*, especially in highly conserved genes. However, there was greater homology with the more closely related *Lucilia sericata* transcriptome assembled in 2010.[15] The sex-specific markers from previous research were also observed in the immature stages.

In conclusion, genomic tools can be used to improve precision and accuracy of mPMI estimates derived from this species. In addition, this study demonstrated that this species of fly also has sexually dimorphic temporal patterns of gene expression throughout immature development, and that these genes share some homology with those found in other organisms. Furthermore, this work suggested that males develop slightly faster than females, and highlight a need for the inclusion of the sex of the individuals in creating developmental profiles. As the insects age, there is a concurrent increase in variation in terms of the relationship between size or length and time. The development of sex-specific growth curves would provide one additional explanation of that variation and, therefore, increase estimate precision.

References:

1. Goldsmith, M.R. and A.S. Wilkins, Molecular model systems in the Lepidoptera 1995, New York: Cambridge University Press.
2. Lima-Catelani, A.R., C.R. Ceron, and H.E. Bicudo, Variation of genetic expression during development, revealed by esterase patterns in Aedes aegypti (Diptera, Culicidae). Biochemical Genetics, 2004. 42(3-4): p. 69-84.
3. Tarone, A.M. and D.R. Foran, Gene expression during blow fly development: improving the precision of age estimates in forensic entomology. J Forensic Sci, 2011. 56 Suppl 1: p. S112-22.
4. Tarone, A.M., K.C. Jennings, and D.R. Foran, Aging blow fly eggs using gene expression: a feasibility study. J Forensic Sci, 2007. 52(6): p. 1350-4.

 ** Presenting Author*

5. Marchini, D., *et al*., cDNA sequence and expression of the ceratotoxin gene encoding an antibacterial sex-specific peptide from the medfly Ceratitis capitata (diptera). Journal of Biological Chemistry, 1995. 270(11): p. 6199-204.
6. Jiang, M., *et al*., Genome-wide analysis of developmental and sex-regulated gene expression profiles in Caenorhabditis elegans. Proceedings of the National Academy of Sciences of the United States of America, 2001. 98(1): p. 218-23.
7. Barmina, O., *et al*., Sex- and segment-specific modulation of gene expression profiles in Drosophila. Developmental Biology, 2005. 288(2): p. 528-44.
8. Sanchez, L., N. Gorfinkiel, and I. Guerrero, Sex determination genes control the development of the Drosophila genital disc, modulating the response to Hedgehog, Wingless and Decapentaplegic signals. Development, 2001. 128(7): p. 1033-43.
9. Shen, J., *et al.,* Identifying sexual differentiation genes that affect Drosophila life span. BMC Geriatrics, 2009. 9: p. 56.
10. McQuilton, P., S.E. St Pierre, and J. Thurmond, FlyBase 101 — the basics of navigating FlyBase. Nucleic Acids Res, 2012. 40(Database issue): p. D706-14.
11. Ullerich, F.H., [Identification of the genetic sex chromosomes in the monogenic blowfly Chrysomya rufifacies (Calliphoridae, Diptera)]. Chromosoma, 1975. 50(4): p. 393-419.
12. Ullerich, F.H., Production of male and female offspring in the strictly monogenic fly Chrysomya rufifacies after ovary transplantation. Naturwissenschaften, 1977. 64(5): p. 277-8.
13. Ullerich, F.H., Die genetische Grundlage der Monogenie beider Schmeilfliege Chrysomya rufifacies (Calliphoridae, Diptera). Molecular & General Genetics, 1973. 125: p. 157-172.
14. Peterson, R.D., J.W. Mackley, and A. Candido, Sugar Feeding by Adult Screwworms (Diptera: Calliphoridae) and Its Effect on Longevity and Oocyte Maturation. Annals of the Entomological Society of America, 1987. 80(2): p. 130-135.
15. Sze, S.H., *et al*., A de novo transcriptome assembly of Lucilia sericata (Diptera: Calliphoridae) with predicted alternative splices, single nucleotide polymorphisms and transcript expression estimates. Insect Molecular Biology, 2012. 21(2): p. 205-21.

Forensic Entomology, Transcriptome, mPMI Estimate

 ** Presenting Author*