

KANSAS STATE
UNIVERSITY
Division of Biology Presents:

**A Single Amino Acid Substitution in a Novel Secreted Protein Specifies
Variation in a Rapidly Evolving Male Reproductive Structure**

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Understanding the molecular mechanisms that specify phenotypic diversity across species is one of the principal goals of evolutionary developmental biology. The male terminalia of the *Drosophila melanogaster* species complex— *D. melanogaster* (mel), *D. simulans* (sim), *D. sechellia* (sec), and *D. mauritiana* (mau)— provide a powerful system in which to study these mechanisms. Males of these species have evolved novel reproductive structures called the epandrial posterior lobes (ePLs), which are essential for successful copulation and vary dramatically in size and shape among the four species. Using genetic mapping and functional tests, we identified a previously uncharacterized gene, Goldilocks (Glds), that negatively regulates ePL size. The Glds protein possesses a signal peptide (SP) at the N-terminus, and we show via a cell-culture secretion assay that the SP is both necessary and sufficient for secretion in all four species. Interestingly, we found that a single, unique amino acid (AA) substitution in the mau SP directs a significantly greater rate of Glds secretion compared to the other species. We performed CRISPR to introduce the unique AA for mau SP into the mel allele of Glds and found a significant decrease in ePL size, consistent with the results of our functional genetic tests that show Glds is a negative regulator of ePL growth. In addition, we performed an in silico protein-protein interaction screen to identify potential interactors of Glds and identified three strong candidates that are expressed in the developing male genitalia. One of these, FMRFaR, is a candidate cell surface receptor that may bind Glds. Deletion of FMRFaR results in a significant decrease in ePL size, which suggests a negative interaction between the Glds ligand and the FMRFaR receptor.

If you would like to visit with Dr. JP Masly, please contact Dr. Gavin Rice at gavinrice@ksu.edu.

Coffee & snacks served preceding the seminar in Ackert Hall, Room 225