form that exits humans and infects mosquitoes, with the compound kills them and a sub-lethal dose blocks their transmission to Anopheles mosquitoes. This establishes plasmepsin V as an essential protein in gametocytes, in addition to the asexual stage, and an attractive drug target for reducing malaria transmission. We have used the inhibitor to obtain diffractable crystals and solved the structure of liganded plasmepsin V to 2.37 Angstroms. This provides a clear basis for the strict requirements for substrate and inhibitor binding of this protease, and unveiled both a plant-like fold and a malaria-specific helix-turn-helix motif that are unique to plasmepsin V and likely to be important in its function in cleavage of effector substrates for export.

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THE PIRNA PATHWAY AND STRESS IN *ANOPHELES STEPHENSI*

Vanessa M. Macias[1], William A. Dunn III[2], Anthony A. James[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Yale University, New Haven, CT, United States

Stress-induced mobilization of transposons is well-documented in many organisms. The piRNA pathway is an RNA interference pathway responsible for repressing transposon mobilization in germ-line tissues of the fruit fly, ***Drosophila melanogaster***. The genes encoding the components of the piRNA pathway, Piwi, Aubergine (Aub) and Argonaut 3 (Ago3), were identified and characterized in the malaria vector mosquito, ***Anopheles stephensi***. Preliminary experiments show that they are induced in embryos following short-duration heat stress. Current experiments are designed to assay the effects of prolonged heat and cold stress on the expression levels of the mosquito orthologs of the heat-shock protein genes, hsp70 and hsp90, as well as Piwi, Aub and Ago3 and putative endogenous transposon transcripts identified from ***An. stephensi*** RNA sequencing data. Additionally, mosquitoes mutant for Piwi, Aub and Ago3 are being generated using Cas9-mediated site-specific genome targeting that will be tested for a phenotype affecting the temperature stress response. The results of this work are expected to inform the development of transposon-based gene-drive systems for introgressing beneficial traits into vector mosquitoes.

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A KEY REPRODUCTIVE GENE INFLUENCES *PLASMODIUM* DEVELOPMENT IN THE MAJOR MALARIA VECTOR *ANOPHELES GAMBIAE*

Perrine Marcenac[1], Adam South[1], W Robert Shaw[1], Emily Lund[1], Abdoulaye Diabate[2], Serge Yerbanga[2], Thierry Lefevre[3], Flaminia Catteruccia[1]

[1]Harvard TH Chan School of Public Health, Boston, MA, United States, [2]Institut de Recherche en Sciences de la Sante/Centre Muraz, Bobo-Dioulasso, Burkina Faso, [3]UMR MIVEGEC, Montpellier, France

In the major malaria vector ***Anopheles gambiae***, male-female molecular interactions following mating are important determinants of fertility and fecundity. Intriguingly, an increasing amount of evidence points to reproductive processes playing an important role in ***Plasmodium*** parasite development. Male transfer of the steroid hormone 20-hydroxyecdysone (20E) during mating activates the transcription of a ***Mating-Induced Stimulator of Oogenesis*** (***MISO***) gene that transduces the mating signal into an increase in egg development. Silencing ***MISO*** by RNA interference reduces egg development to levels observed in virgin females. This phenotype is caused in part by improper release of 20E from the mating plug in the absence of MISO, leading to disregulation of genes important for oogenesis. In particular, silencing ***MISO*** reduces expression of yolk protein precursors (YPPs) and impairs lipid accumulation in the oocyte. Previous research shows that the same YPPs essential for lipid accumulation in the developing mosquito egg help parasites escape the immune system. We show evidence that MISO depletion impacts both ***Plasmodium falciparum*** and ***P. berghei*** infection in ***A. gambiae;*** however, the effects in these ***Plasmodium*** species differ, enabling us to further reconstruct the molecular pathways linking egg development and ***Plasmodium*** infection. Our studies suggest ***MISO*** may modulate aspects of mosquito biology that are relevant to anopheline vector competence.

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INVESTIGATING MOSQUITO MOLECULAR FACTORS THAT CONTROL GUT MICROBIOTA VARIABILITY IN *AEDES AEGYPTI*

Sarah M. Short, Octavio C. Talyuli, George Dimopoulos

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

The ***Aedes aegypti*** mosquito midgut microbiota can alter mosquito susceptibility to dengue virus, and understanding factors that shape it could explain field transmission dynamics and contribute to development of novel dengue control strategies. In the current study, we aimed to identify mosquito molecular factors that control bacterial load of the midgut and contribute to within-species variability in gut microbial load. We reared multiple strains of ***A. aegypti*** in a controlled laboratory environment and used culture-dependent and -independent techniques to assess bacterial load in the midguts of sugar and blood fed females from each strain. We then compared genome-wide gene expression in response to blood feeding and bacterial ingestion between two strains showing the greatest difference in microbial load. We identified genes that showed strain-specific patterns of up- or down-regulation and used Gene Ontology and KEGG analyses to identify pathways enriched in a strain-specific manner. Finally, we used RNAi to knock down candidate genes to validate their role in influencing gut microbial load. We found significant variation between strains in gut bacterial load. Our transcriptome analysis revealed that an unexpectedly high number of metabolism-implicated genes were differentially expressed between strains. We also identified strain-specific variation in the mRNA abundance of multiple immunity genes. Preliminary data from RNAi knock down experiments suggests that the immunity gene galectin 1 as well as genes involved in valine, leucine and isoleucine degradation are implicated in controlling proliferation of gut bacteria in a strain-specific manner. Taken together, these data suggest that metabolic activity in the mosquito gut may act to control bacterial load and that variability in metabolic activity has the potential to control within-species variation in gut microbial load.

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A PROTEOMIC-BASED "SHOTGUN" APPROACH OPENS NEW PERSPECTIVES TO MOSQUITO AGE-GRADING

Immacolata Iovinella[1], Beniamino Caputo[1], Elena Michelucci[2], Francesca Dani[2], **Alessandra della Torre**[1]

[1]University of Rome, Sapienza, Rome, Italy, [2]University of Florence, Florence, Italy

Information on age structure of wild mosquito populations is fundamental to assess impact of control measures and vectorial capacity of species implicated in transmission of malaria or arboviroses. Transcriptional studies on major disease vectors, such as ***Anopheles gambiae*** and ***Aedes aegypti***, have highlighted age-related variations in some genes which have been exploited to develop quantitative reverse transcriptase-PCR age-grading methods. However, due to low RNA stability, these qRT-PCR approaches require a careful manipulation and preparation of samples and relatively high-tech and expensive equipment. In this work we applied a proteomic-based "shotgun" approach to identify and quantify proteins from carcasses of laboratory reared Aedes albopictus belonging to six different age-groups and different physiological stages. Proteins were extracted from heads and thoraxes and the same total protein content of each group was processed for a nanoLC-nanoESI-MS/MS analysis on an Ultimate 3000 HPLC coupled to a LTQ Orbitrap mass spectrometer. Protein intensities across samples was evaluated using a LFQ (label-free quantitation) method. Approximately 600 proteins/age-group were identified, 4 of which (i.e. an hemocyanin protein; an insect cuticle