

of >1.8. *E. coli* ATCC 25922 gave a reliable score (>2) every time, when matched against the in-built database of Biotyper. Thus, MALDI-TOF MS appears to be a pragmatic technique for accurate and rapid identification of mosquito species. The database can be further expanded to include larvae and pupae stages and also species from different geographical regions.

849

WANGA IN CELL CULTURE: TOOLS FOR STUDYING ASSOCIATIONS BETWEEN ANOPHELES AND WOLBACHIA

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wAnga is to date the first and only strain of *Wolbachia* that has been identified in natural populations of *Anopheles gambiae* mosquitoes, the principle vectors of malaria (Baldini et al., (2014) Nat. Comm. 5:3985). *wAnga* is negatively associated with natural *Plasmodium falciparum* coinfection (Shaw et al., (2016), Nat. Commun. 7:11772), but there is no evidence that it can cause cytoplasmic incompatibility, limiting its potential use in vector control. Moreover, *wAnga* is also found at very low densities in the sister species *An. coluzzii*. We hypothesize that *wAnga*'s low titers may limit its ability to cause reproductive phenotypes in its host. We have established *wAnga* infections in *An. coluzzii* Mos55 cells and *Drosophila melanogaster* S2 cells, and are using cell culture to facilitate the study of this bacterium. We will make comparisons between *wAnga*'s ability to grow in *An. coluzzii* Mos55 cells and *D. melanogaster* S2 cells, which could provide valuable insight regarding the hospitability of the anopheline environment for *Wolbachia*. We are also working to transfer the *wAnga* strain via cell culture into other anopheline species. We hypothesize that an *Anopheles*-specific strain is more likely to be successfully transferred and stably maintained in other anophelines than *Aedes*- or *Drosophila*- specific *Wolbachia* strains, which have been met with recalcitrance in *Anopheles* thus far. Furthermore, we are striving to manipulate titers of *wAnga* using cell culture systems, with the goal of translating these findings into *in vivo* tools for the study of *wAnga*-induced host phenotypes. We are also taking advantage of the ability of cell culture to facilitate whole genome sequencing efforts by improving the capacity to obtain high purity DNA in higher ratios. This research will provide new knowledge to the field of innovative vector control against *Anopheles* mosquitoes, specifically towards assessing the potential of *Wolbachia* as a vector control agent for malaria transmission.

850

THE ADULT Aedes Aegypti MOSQUITO MIDGUT PERITROPHIC MATRIX PROTEOME

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The *Aedes aegypti* mosquito is the principle vector of arboviruses such as dengue, chikungunya, yellow fever and Zika virus. These arboviruses are transmitted during adult female mosquito blood feeding. While these viruses must transverse the midgut to be transmitted, the blood meal must also reach the midgut to be digested and subsequently used for egg development. However, this is a high-risk, high-reward process, as aggregation of blood meal metabolites can be toxic to the female mosquito midgut. Understanding the mechanisms that allow for midgut protection may provide novel molecular-based control strategies for mosquitoes and mosquito-borne diseases. The midgut peritrophic matrix (PM), a semipermeable extracellular layer that forms in response to blood feeding and separates midgut epithelial cells from the blood bolus, may serve as one such mechanism. While previous studies suggest the PM is comprised of 20-40 major proteins, only two have been identified to date. We conducted a mass spectrometry based proteomic analysis to identify

proteins that comprise the adult female *Ae. aegypti* midgut peritrophic matrix. Altogether, 474 unique proteins were identified, with 115 predicted secreted proteins. Of these, 57 were associated with catalytic activity, 20 were conserved hypothetical proteins, 8 were hypothetical proteins and 17 were of salivary gland origin. Most interestingly, we identified a conserved hypothetical protein of unknown function with characteristics similar to known peritrophic matrix proteins. This protein may be integral for midgut protection from blood meal derived toxicity, and serve as a novel target for vector control.

851

ELUCIDATING THE ROLE OF LIPOLYTIC PATHWAY IN MOSQUITO REPRODUCTION AND PLASMODIUM FALCIPARUM TRANSMISSION

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Female *Anopheles* mosquitoes undergo a number of blood feeding cycles on a vertebrate host in order to produce multiple egg batches during their life span, and these obligatory steps are exploited by *Plasmodium* parasites for their own transmission. Blood feeding is therefore a critical step for both mosquito reproduction and parasite transmission that could be exploited to impact malaria dynamics in endemic areas, especially as these two processes are temporally and physiologically coupled. Previous studies revealed a correlation between blood meal digestion and major changes in transcriptional profiles of metabolic genes involved in lipid biosynthesis, transport, and breakdown, suggesting the occurrence of *de novo* lipid synthesis triggered by blood feeding and followed by lipid mobilization. Here, we aim to elucidate the specific role of blood meal-derived lipids (and/or of lipids synthesized *de novo* after a blood meal) in *Anopheles gambiae* reproduction and *Plasmodium falciparum* parasite development in mosquito stages. To address this, we initially performed targeted lipidomic analyses of various mosquito tissues after a blood meal. Our analyses reveal a coordinated accumulation and depletion of major lipid classes across key mosquito tissues during blood meal digestion, reflective of an engagement of lipogenic and lipolytic pathways. RNA interference (RNAi) against triglyceride (TAG) lipase and associated proteins, involved in lipolytic breakdown of TAGs to yield free fatty acids and diacylglycerol (DAG) identifies lipid mobilization as central in determining reproductive success of the main malaria vector. Specifically, TAG-lipase inhibition, significantly impairs egg development and abolishes fertility, and upon a *P. falciparum* infectious feed had no apparent impact on the number of oocysts per midgut. While further characterization is underway, this study identifies the regulation of TAG/DAG equilibrium as critical for achieving reproductive success of *Anopheles* mosquitoes that could be exploited to control mosquito population and reduce malaria transmission.

852

A FEMALE REPRODUCTIVE PROTEIN AFFECTS THE INTERACTION BETWEEN ANOPHELES GAMBIAE MOSQUITOES AND PLASMODIUM FALCIPARUM PARASITES

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In *Anopheles gambiae*, male-female molecular interactions following mating are important determinants of female reproductive fitness. *Anopheles* oogenesis and *Plasmodium* parasite development are both temporally and physiologically tied, and an increasing amount of evidence points to reproductive processes playing an important role in

Plasmodium falciparum development. Transfer of the steroid hormone 20-hydroxyecdysone (20E) from males to females during mating induces the expression of the gene *Mating-Induced Stimulator of Oogenesis* (*MISO*) in a number of *Anopheles* species and forms a complex with this factor. We find that species that have evolved this mating system have high tolerance to *P. falciparum* infection, and that depletion of *MISO* via RNA interference induces a significant fecundity cost in infected females. We hypothesize that *MISO* has evolved as a key factor to maintain female fitness in the face of *Plasmodium* infection. Our studies suggest male-transferred 20E may modulate aspects of female mosquito biology that are relevant to anopheline vector competence, which could have immense implications for *P. falciparum* transmission. Understanding how male-female molecular interactions following mating affect *P. falciparum* infection in the mosquito vector is key to the development of future vector control tools.

853

HIGHLY CONSERVED PATTERN OF INTERGENOMIC SEQUENCE VARIATION IN INTERNAL TRANSCRIBED SPACER 2 (ITS2) IN ANOPHELES SUBPICTUS SPECIES A ACROSS WIDELY DISTRIBUTED POPULATIONS

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Anopheles subpictus s.l. has emerged as a malaria vector in India and Sri Lanka which consists of at least four cryptic species. Ribosomal DNA (rDNA) is extensively used for discrimination of cryptic species that is considered as a gold standard marker for taxonomic resolution of closely related species. Ribosomal DNA is considered highly conserved within an interbreeding population due to concerted evolution acting on this multigene family and therefore has been extensively used for identification of closely related species especially for the discrimination of cryptic species that are morphologically indistinguishable. It is assumed that all copies of rDNA within an individual are identical, however there are some report of presence of intergenomic sequence variation in rDNA. This phenomenon may affect the species diagnostic value of rDNA. We sequenced internal transcribed spacer rDNA (ITS2) of *Anopheles subpictus* species A from different geographical populations from Indian subcontinent (Alwar 27.5° N, 76.6°E; Jodhpur 26.2° N, 73.0° E; Delhi 28.7° N, 77.1° E; Kheda 22.9° N, 72.9° E; Ranchi 23.3° N, 85.3° E; Chilka Lake 19.8° N, 85.4° E; Jaffna, Sri Lanka 9.6° N, 80.0° E) and discovered constant pattern of intergenomic variation in rDNA of *An. subpictus* species A with presence of two types of sequences. Cloning and sequencing of ITS2 from individual mosquitoes revealed presence of two types of sequences differing by indel of 12 bases. However, all populations showed identical intergenomic sequence variation with presence of these two types of sequences. We infer that intergenomic sequence variation will not affect species diagnostic value of ribosomal DNA.

854

DISCOVERY OF A NOVEL MOSQUITO JUVENILE HORMONE BINDING PROTEIN ISOLATED FROM THE YELLOW FEVER MOSQUITO, Aedes Aegypti

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Juvenile hormone (JH) is a key regulator of insect metamorphosis and reproductive development. JH binding protein (JHBP) is responsible for transporting JH to target tissues while protecting it from degradation and regulating its bound concentration. Although JHBP has been identified and physically characterized in agricultural pest insects (Lepidoptera), specific function and structure of JHBP remains unknown in medically-important

insects including mosquitoes. Here, we are the first to describe a new type of JHBP isolated from the yellow fever mosquito, *Aedes aegypti* (nJHBP). nJHBP is a member of the odorant-binding protein family (OBP) and is related to the D7 proteins found in mosquito saliva. Unlike Lepidoptera JHBP which is found in all life stages, Western blot analysis revealed that nJHBP circulates only in the hemolymph of pupal and adult mosquitoes. Binding studies using isothermal titration calorimetry showed that nJHBP binds JH II and JH III with high affinity. However, nJHBP did not interact with the eicosanoid fatty acid ligands of the D7 proteins or JH analogues lacking the epoxide group such as methoprene. nJHBP was crystallized in the presence of JH III and found to have a two OBP domain structure. A single molecule of JH III was found in the N-terminal domain binding pocket that is enclosed by a cap structure derived from the C-terminal domain. The lack of clear structural path to the binding site suggests that its binding mechanism is analogous to unrelated Lepidoptera JHBP. Relative quantification of nJHBP mRNA in female mosquitoes indicated nJHBP expression reaches its highest level at 12 hour post-eclosion, corresponding to a reported peak of JHIII synthesis. nJHBP expression level declines after 12 hours and remains low after blood-feeding, which also follows the expression pattern of JHIII. Altogether, these results document a discovery of a novel JHBP in mosquitoes. This finding has a major implication for understanding how JH is regulated in mosquitoes and future studies will aim to further understand the role of nJHBP in regulating JH and its mechanism of action in delivering JH to target sites.

855

VECTORBASE: DATABASE FOR POPULATION BIOLOGY AND OMICS DATA QUERY, BROWSE AND ANALYSES

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VectorBase (www.vectorbase.org) is a free, web-based bioinformatics resource center (BRC) for invertebrate vectors of human pathogens, funded by NIAID/NIH. This database is the 'home' of genomes of arthropod vectors and pests (e.g., *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* among other species in Diptera, Hemiptera, Phthiraptera, and Acari), phylogenetically related species, and one intermediate host (the snail, *Biomphalaria glabrata*). In addition to these 40 genomes, it also has transcriptomes, proteomes and population data for an even wider list of species. The population biology data includes lab and field collected information, both from genotypes and phenotypes, covering any biology, ecology, or behavior trait, and even insecticide resistance and population abundance. In addition to the data imported from external databases or directly submitted by users, VectorBase also generates and computes primary data. Over its 13 years of existence, the discovery and interpretation of hosted data has been used for basic and translational research, as expressed in numerous scientific publications, using data in new or re-purpose analyses, descriptions and hypotheses testing. Raw and process data can be exported or download in a variety of different formats, visualized, browsed, queried and analyzed with the site tools or any other external tools. Because VectorBase data, tools and resources are updated every two months, this presentation will highlight this last year major additions. The website has extensive documentation resources for new and experienced users including tutorials, video tutorials (YouTube/youku), practice exercises, answer keys and sample files. In addition to our standard (in-person) workshops, this year we started to offer live webinars. Thesis or publications using this database, are kindly ask to reference the paper or papers where the data was originally published and VectorBase most recent paper, as explained in the website under the "Help" navigation tab. To contact us send a message to info@vectorbase.org.