2017 10th Arthropod Genomics Symposium

June 8 – June 11, 2017

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Arthropod Genomics Center
Kansas State University

UNIVERSITY OF NOTRE DAME
ECK INSTITUTE FOR GLOBAL HEALTH

2017 10th Arthropod Genomics Symposium
June 7 – 11, 2017

Schedule of Events

Thursday, June 8
Smith Ballroom Concourse, The Morris Inn

5:30 – 7:00pm Registration

6:00 – 7:30pm Buffet Dinner

7:30 – 7:45pm Opening Remarks
Session Chair: Nora Besansky, University of Notre Dame

7:45 – 8:45pm Keynote Lecture – George Christophides, Imperial College, London
"Cartography-inspired journeys into mosquito genomics landscapes"

8:45 – 10:30pm Welcome Reception

Friday, June 9
University of Notre Dame Conference Center McKenna Hall

7:30 – 8:00am Odd numbers poster set up
100/104 and 112/114 McKenna Hall

7:30am Continental Breakfast

8:00 – 10:00am i5K/Emerging Genomes Session
Session Chair: Sue Brown, Kansas State University

Denis Tagu, Rennes, Inra, France – "*Integrative genomics and gene networks for studying phenotypic plasticity in the pea aphid*"

Gregg Thomas, Indiana University – "*Evolution of the genes and genomes of 76 arthropod species*"

Charles Brockhouse, Creighton University – "*Progress and Applications of the Simuium Genome Project*"

Zachary Cohen, University of Wisconsin-Madison – "*Comparative phylogenetics and genome evolution of the global agricultural pest, Colorado potato beetle*"

Scott Geib, USDA-ARS – "*Characterization of genetic sexing traits in established genetic sexing lines for development of novel genetic sexing systems*"

10:00 – 10:30am Break

10:30 – 12:20pm Ecological/Population Genomics Session
Session Chair: Scott Small, University of Notre Dame

Megan Fritz, University of Maryland – *"Has selection associated with Bt crops impacted the genome of agricultural pest, Heliothis virescens?"*

Alistair Miles, The Wellcome Trust Centre for Human Genetics – *"Genome variation in 1,142 malaria mosquitos"*

Meredith Doellman, University of Notre Dame – *"Divergent natural selection on eclosion time variation affects genome-wide patters of geographic and host related differentiation for apple and hawthorn host races of Rhagoletics pomonella."*

Nicholas Negre, University of Montpellier – *"Genomic, transcriptomic and epigenomic variations in response to host-plant in the agricultural pest Spodoptera frugiperda (Lepidoptera: Noctuidae)"*

12:20 – 2:00pm Lunch on own

2:00 – 3:30pm Dessert, coffee, and Poster Session I
Odd numbered posters, 100/104 and 112/114 McKenna Hall

3:30 – 5:15pm Functional Genomics Session
Session Chair: Molly Duman Scheel, Indiana University School of Medicine – South Bend

Sara Cherry, University of Pennsylvania – "*Using functional genomics to explore arbovirus-host interactions*"

JoAnna Chiu, UC-Davis – "*Leveraging genomic approaches to study seasonal biology of Drosophilia suzukii*"

Marc Halfon, University at Buffalo-State University of New York – "*REDfly and SCRMshaw: powerful tools for insect regulatory genomics*"

Agnes Ayme-Southgate, College of Charleston – "*Regulation of alternative splicing during the nurse-forager transition in Apis mellifera*"

5:15pm Remove odd numbered posters

5:30 – 7:30pm BBQ Cookout (weather permitting) and Roundtable Discussion "NGS sequencing and tools applied to arthropods"
South Dining Hall

7:30 – 9:00pm Entertainment and Social, Bill O'Hayer and Company
South Dining Hall

Saturday, June 10
University of Notre Dame Conference Center, McKenna Hall

7:30 – 8:00am Even numbers poster set up
100/104 and 112/114 McKenna Hall

7:30am Continental Breakfast

8:30 – 10:30am Vector Genomics Session
Session Chair: Rebecca Love, University of Notre Dame

Zach Adelman, Texas A&M University – "*Comparative transcriptomics across seven members of the genus Aedes*"

Lyric Bartholomay, University of Wisconsin-Madison – "*RNAi interference: receptive and unresponsive spaces and places in the body of a mosquito*"

Christopher Holmes, University of Cincinnati – "*Dynamics between Dehydration, Carbohydrate Metabolism, and Blood Feeding in Mosquitoes Revealed by Combined Transcriptomic and Metabolomic Analyses*"

Seth Redmond, Broad Institute of Harvard and MIT – "*Structural variant detection by read-cloud sequencing in Aedes aegypti*"

10:30 – 11:00am Break

11:00 – 12:40pm Metagenomics Session
Session Chair: Sherry Miller, Kansas State University

Rita Rio, Western Virginia University – "*Microbiota integration in tsetse biology*"

Richard Kuhn, Purdue University – "*Lipidomic and Proteomic Analyses of Flavivirus – Vector Interactions*"

Robert Glaser, Wadsworth Center, NY State Dept. of Health – "*Mapping QTLs in Culex quinquefasciatus that control the density of bacterial symbiont Wolbachia pipientis*"

W. Allen Miller, Iowa State University – "*Discovery of Known and Novel Viral Genomes in Soybean Aphid by Deep Sequencing*"

12:50 – 2:00pm Undergraduate Roundtable Discussion and Lunch (Pre-registered)
McKenna Hall

2:00 – 3:30pm Dessert, coffee, and Poster Session II
Even numbered posters, 100/104 and 112/114 McKenna Hall

3:30 – 5:10pm Sensory Genomics Session
Session Chair: Zainulabeuddin Syed, University of Notre Dame

Zainulabeuddin Syed, University of Notre Dame – "*Evolution of chemical communication in a pest fly, Drosophila suzukii*"

Anupama Dahanukar, UC-Riverside – "*Functional analysis of insect sweet taste receptors*"

Keshava Mysore, Indiana School of Medicine-South Bend – *"A Screen for Chemosensory Gal4 Drivers that function in Multiple Dipteran Insects"*

Carol Lee, Center of Rapid Evolution (CORE) and Department of Integrative Biology, University of Wisconsin – *"Evolutionary History of Chemosensory – Related Gene Families across the Arthropoda, with special focus on the Pancrustacea"*

5:15pm Remove even numbered posters

George B. Craig Jr. Memorial Banquet and Lecture
Main Building (Dome) Rotunda

6:30 – 7:30pm Dinner, Pre-registered only

7:30 – 8:30pm Lecture, Michael MacDonald, ScD – *"Public Health Entomology and Vector Control in the Greater Mekong Sub-region"*
Open to public

Abstract Table of Contents

Platform/Oral Presentations

Posters

13. **Biocuration and improvement of the *Diaphorina citri* draft genome assembly with long reads, optical maps and long-range scaffolding** Surya Saha.....

14. **Sequencing of an extremophilic icebug, *Galliosiana yuasai*** Felipe Simao.....

15. **The sequence of a W-chromosome for the diamondback moth, *Plutella xylostella,* revealed with long-read genome assembly and single chromosome sequencing** Sam Whiteford.....

16. **Transcriptome analysis and expression of sensory genes in *Aedes aegypti* associated with ultrasonic sound treatment** Jewon Jung.....

17. **Genome of the parasitic wasp *Diachasma alloeum,* an emerging model for ecological speciation and transitions to asexual reproduction** Eric Tvedte.....

18. **Metabolomics of diapause in *Aedes albopictus*** Zachary Batz.....

19. **Regulation of population size in facultative endosymbionts of the pea aphid** Serena Zhao.....

20. **Untangling the transmission dynamics of primary and secondary vectors of *Trypansoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units** Carolina Hernández.....

21. ***Anopheles* genome assembly improvements guided by evolution** Max Alekseyev.....

22. **Dynamic of *Spodoptera frugiperda* chromatin histones marks regarding different life stages** Sandra Nhim.....

23. **Aphid Transcriptomic Analysis and the Development of Integrated Pest Management Strategies in Greenhouse Crops** Yonathon Uriel.....

24. **Proteomic analysis of the midgut and malpighian tubules of laboratory and field strains of *Aedes aegypti* from Colombia** Angelica Aponte Hincapie.....

25. **The male fertility gene *kl-3* is linked to the Y chromosome of the kissing bug *Triatoma infestans*** Leonardo Koerich.....

26. **Genome sequencing, assembly and annotation of the spider *Dysdera silvatica* Schmidt, 1981 (Araneae, Dysderidae). A resource for the study of an adaptive radiation in the canary islands** Jose Sánchez.....

27. **From single genomics to population genomics, iterative improvement of genome annotation for multiple vector species at VectorBase** Dan Lawson.....

28. **VectorBase: A bioinformatics resource for invertebrate vectors and other organisms related with human diseases** Gloria Giraldo-Calderon.....

29. **Improvements to the *Lucilia cuprina* draft genome and transcriptome** CA Anstead

30. **Differential transcriptomic profiles in honey bee *Apis mellifera* workers under brood rearing suppressed condition** Kyungmun Kim.....

31. **The methylome of the marbled crayfish, a novel model system for epigenetics** F. Gatzman.....

32. **Light manipulation of mosquito behavior: Acute and sustained photic suppression of biting in the *Anopheles gambiae* malaria mosquito** Samuel Rund.....

33. **Genome-Wide Profiling of Diurnal Rhythmic Gene Expression in the Water Flea *Daphnia Pulex*** Samuel Rund.....

34. **Using Engineered yeast expressing interfering RND larvicides to control *Aedes aegypti*** Limb Haparai.....

35. **The *Gammarus* genome project: building a consortium for challenging the genome diversity of a crustacean family of ecotoxicological importance** Davide Degli Esposti...

36. **Biosurveillance of Alien Forest Enemies (bioSAFE) – creating new genomic tools to meet the challenges posed by forest alien invasives** Amanda Roe.....

37. **Two sand fly genomes: *Phlebotomus papatasi* and *Lutzomyia longipalpis*** Mary Ann McDowell.....

38. **Community annotation across 26 non-model arthropod species** Monica F. Poelchau....

39. **Chromosome level assembly of the *Apis dorsata* and *Apis florea* genome** Hung Nguyen...

40. **HymenopteraMine at Hymenoptera Genome Database: An Efficient and Customizable Data Mining Resource for Improved Genomic Analysis** Darren Hagen.....

41. **Targeted next-generation sequencing of detoxification genes in *Culex pipiens* complex mosquitoes: Discovery of SNPs and copy number variants associated with insecticide resistance** Linda Kothera.....

42. **Analysis of insect *Kirre/Roughest* and *Sticks and stones/Hibris* paralogues groups reveals concurrent gene duplication events for both ligand and receptor genes in Diteran lineages** Ronald Bayline.....

43. **Investigation of Neuropeptide F as a Novel Insecticide Target** Kaitlin Frei.....

44. **Islands with moderate genetic differentiation and small effective population sizes of the malaria vector *Anopheles gambiae:* field sites for evaluating transgenic drive?** Rachel Wiltshire.....

45. **Serotonin receptors as druggable targets** Michelle Ngai.....

46. **Delivery of Gene BioTechnologies to Plants: Pathogen and Pest Control** Jackie Metz...

47. **Method for Detecting Binding Efficiencies of synthetic Oligonucleotides: Targeting Bacteria and Insects** Wayne Hunter.....

48. **Genetics and genomics of host specificity in aphid parasitoids** Alisha Johnson.....

49. **On the Origin of the W chromosome in Lepidoptera: Insights from the Z chromosome** Anna Volenikova.....

50. **Systems biology resources for *Diaphorina citri,* a vector for the Citrusgreening disease** Mirella Flores-Gonzalez.....

51. **Genome assembly for experimental evolution in the pigeon louse C. *columbae*** James Baldwin-Brown.....

52. **Use of genome and transcriptome information for the development of male-only strains of the New World screwworm** Max Scott.....

53. **Metabolic communication: transporters play a key role in the tsetse-*Wigglesworthia* nutrient exchange** Miguel Medina Muñoz.....

54. **Genetic mechanism underlying adaptive variation of bumblebee mimetic coloration** Li Tian.....

55. **Retention of duplicated long-wavelength opsins in mosquito lineages by positive selection and differential expression** Gloria Giraldo-Calderon.....

56. **Genome assembly of the Luna Moth, *Actias luna* (Lepidoptera: Saturniidae)** Deborah Triant.....

57. **Gene expression profiles of gene associated with silk production in Dysdera spiders** Mark Alonzo.....

58. **Using novel, low-cost sequencing technologies to increase genomic resources for agricultural pests** Erin Scully.....

59. **Transcriptome assembly and differential expression analysis of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in response to Bt intoxication** Zixiao Zhao...

60. **A comprehensive bioinformatics guideline for conducting Genome Wide Association Studies (GWAS) in haploid non-model organisms** Sarthok Rahman.....

61. **Islands with moderate genetic differentiation and small effective population sizes of the malaria vector *Anopheles gambiae:* field sites for evaluating transgenic drive?** Rachel Wiltshire.....

62. **Targeted next generation sequencing of detoxification genes in *Culex pipiens* complex mosquitoes: Discovery of SNPs and copy number variants associated with insecticide resistance** Linda Kothera.....

63. **Investigation of Neuropeptide F as a Novel Insecticide Target** Kaitlin Frei.....

64. **GPCR Targeted Insecticide Design for Control of Vector Mosquitoes Transmitting Dengue and Zika** Zoe Loh.....

65. **The making of a pest: Insights from the evolution of chemosensory receptor families in a pestiferous and invasive fly, *Drosophila suzukii*** Paul V. Hickner.....

Platform/Oral Presentations

KEYNOTE LECTURE

Cartography-inspired journeys into mosquito genomics landscapes

George Christophides
Imperial College, London

The availability of large volumes of genomics data has created expectations for deep and rapid understanding of life and its evolution. Yet these data cannot be easily visualised and analysed by the broader scientific community and more so in a holistic and integrative manner. Inspired by traditional methods of cartography, we have developed a pipeline for the generation of pseudo-maps of mosquito genomics data, including expression, evolution and variation. These maps allow unprecedented and intuitive journeys into the mosquito genomics landscapes and development of new understandings and testable hypotheses. They can also be adapted to fit other data types and taxa.

i5K/EMERGING GENOMES

Integrative genomics to decipher phenotypic plasticity in aphids

V. Wucher, F. Legeai, L. Bourneuf, T. Derrien, A. Gallot, S. Hudaverdian, S. Jaubert-Possamai, N. Leterme-Prunier, J. Nicolas, H. Seitz, A. Siegel, S. Tanguy, G. Le Trionnaire, D. Tagu

Phenotypic plasticity is an adaptive process widespread in Insects. This involves the capacity of embryos to sense external local environmental cues during their development with alternative routes providing a final phenotype adapted to those new cues. In our lab, we describe and analyse the gene expression regulation rules during the establishment of the phenotypic plasticity of the reproductive mode in aphids due to the change of the photoperiod in the fall. The development of sexual and asexual embryos that differ mainly by their production of haploid meiotic gametes or diploid non-recombinant gametes is thus compared. We developed an integrative genomics approach that aims to define all the functional DNA elements in the formation of alternative morphs. We thus combine genomics (nucleic acid sequencing), bioinformatics and mathematic modelling to construct gene networks describing and modelling the molecular processes acting in the phenotypic plasticity of the reproductive mode. We annotated functional DNA elements (open chromatin, long non-coding RNAs, microRNAs, mRNAs), predicted their putative interactions and modelled their network functioning. We identified two major biological processes as important signatures of these networks: “oogenesis” and “central nervous system”. We analysed more deeply some of the candidates to fill the gap between prediction, hypotheses and biological observations.

Evolution of the genes and genomes of 76 arthropod species

Gregg Thomas
Indiana University

Arthropods are the largest and most diverse eukaryotic phylum on Earth, and have justifiably been the focus of a very large body of research over the past centuries. The advent of whole genome sequencing in the last

decade has opened new avenues of research that are poised to change the way we carry out comparative work on arthropods, and could add significant insight into arthropod evolution. Here we utilize 25 newly sequenced genomes by the i5k pilot project, along with 51 other publicly available genomes, to assess the phylogeny and patterns of molecular evolution across Arthropoda. In all, our 76 species span 22 arthropod orders. Orthology prediction from OrthoDB yielded 38,195 gene families with which we were able to reconstruct the arthropod phylogeny and investigate aspects of insect genome evolution in this phylogenetic context. This includes determination of gene families that are rapidly changing on specific lineages, providing insight into the evolution of several clades of interest. This is one of the largest whole-genome analyses to date and sheds light on several key aspects of arthropod evolution, including characteristics of the last insect common ancestor (LICA).

Progress and Applications of the Simuium Genome Project

Charles Brockhouse on behalf of the Simulium Genome Consortium.
Biology Department, Creighton University, Omaha, NE, 68178, USA

The family Simuliidae (Diptera) is ranked as the second most significant arthropod pest taxon in terms of human/veterinary health and economic impact. Various members of this family create burdens both through mass biting and disease transmission. Onchocerciasis (River Blindness) is the most significant disease transmitted by simuliids, with an impact of over 1 million disability adjusted life years per annum (primarily in sub-Saharan Africa). While difficulties in establishing and maintaining colonies of pest species have precluded genetic studies, wild populations have been the focus of polytene chromosome cytogenetics for over 60 years. The Simulium Genome Consortium has initiated whole genome sequence, transcriptome and proteome studies of pest and model species. Applications include gene-flow studies to establish transmission zones, identification of markers for reproductive state, and characterization of biologically interest genes.

Comparative phylogenetics and genome evolution of the global agricultural pest, Colorado potato beetle

Zachary Cohen[1], Yolanda Chen[2], and Sean Schoville[1]
[1] University of Wisconsin-Madison, Madison, WI; [2] University of Vermont

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say, is a significant agricultural pest on cultivated *Solanum* crops such as potato, tomato, and pepper (Grapputo et al., 2005). The *Leptinotarsa* genus comprises 41 known species distributed from North to South America, with the center of diversity located in Mexico (Jacques 1988). To date, there is no phylogeny on this genus. In order to elucidate the genomic changes associated with the development of CPB as a pest, we compare whole genomes of ten closely related *Leptinotarsa* species from North America and Mexico. A species tree was constructed using these whole genome sequences to provide a tool for further investigation. Using this phylogeny as a backbone, we examine gene family expansion/contraction events and rapid protein evolution tied to the emergence of this successful agricultural pest.

Characterization of genetic sexing traits in established genetic sexing lines for development of novel genetic sexing systems

Scott M. Geib and Sheina B. Sim
USDA-ARS Daniel K Inouye U.S. Pacific Basin Agricultural Research Center, Hilo, HI

Tephritid fruit flies (Diptera: Tephritidae) are a major pest of worldwide economic importance. Their threat to high value agriculture is so extensive that area-wide integrated pest management programs (AW-IPM) are employed to prevent their long-term establishment in the mainland United States. An important component of these AW-IPM programs is the use of the sterile insect technique (SIT) by taking advantage of genetic sexing strains (GSS) that are established for these species. In order to have an effective SIT program, a genetic sexing strain (GSS) needs to be established for the species of interest, which facilitates cost-effective and rapid separation of female and male flies during mass-rearing, and release of male-only sterile flies. Our goal is to identify the genetic basis for the white pupae and temperature sensitive lethal traits in existing GSS, identify the causative mutations in genes causing phenotypic changes in these strains, and develop tools to transfer these traits to other economically important Tephritid species using targeted genome editing approaches. To address these objectives, we employed methodologies that bring together classical genetics with high-throughput sequencing techniques and emerging genome editing technologies. To do so, we leverage the availability of complete and highly contiguous reference genomes and combine QTL mapping, 3' mRNA sequencing, and whole genome resequencing to investigate the possible causative mutation for white pupae and genes that are involved in the pupal color process. These methods combined allowed for extensive sampling and replicates to improve statistical power at a substantial reduction in cost. To validate the function of one of the genes identified through differential expression analysis and whole-genome resequencing, we employed CRISPR/Cas9 targeted mutagenesis to attempt to recreate the mutations in wild-type individuals, screening for phenotype. Once the causative mutations are identified, through comparative genomic analysis (orthology/synteny) to other Tephritid genomes, putative targets in those genomes will be identified to introduce the same GSS mutations into colony lines of those species. In addition to identifying genes associated with genetic sexing traits, through long ranged linked-read (10X) and single molecule (Oxford Nanopore) sequencing, we assess chromosomal re-arrangements (translocations) that confer these traits as sexed link. This will provide the foundation to developing GSS lines for important Tephritid pests as well as other economically important species, which currently are not amenable to male only mass rearing for SIT.

ECOLOGICAL/POPULATION GENOMICS

Has selection associated with Bt crops impacted the genome of agricultural pest, Heliothis virescens?

Megan Fritz
University of Maryland

Heliothis virescens is a primary pest of cotton with a recurring history of insecticide resistance. Most recently, they evolved high levels of phenotypic resistance to pyrethroids in the mid 1990s. One non-synonymous point mutation encoding a Leu to His amino acid change in an alpha subunit of the voltage-gated sodium channel (VGSC) contributed to this resistance in Southern US populations. Yet this mutation comes with a fitness cost, and is only beneficial when pyrethroid pressure is high. In 1996, the first Bt cotton cultivar was commercialized, and transgenic cultivars now dominate the cotton-growing agricultural landscape. H. virescens are now well-managed by Bt cotton and their populations are in decline. Successful management of H. virescens with transgenic cotton, along with the development of new insecticidal chemistries, has resulted in a decline in pyrethroid inputs into the environment. Given the cost of carrying the VGSC resistance allele when pyrethroid pressure is low, we predicted that selection against this allele had taken place. We examined the molecular evidence for a decline in the VGSC resistance allele in archived field-collected populations of H. virescens. Following detection of VGSC allele frequency changes, we tested whether ddRAD-seq enabled genome scanning and outlier analysis could "rediscover" the VGSC locus. Our results indicated that genomic scanning techniques could be used to monitor pest populations for insecticide resistance, even when the gene targets of resistance are unknown.

Genome variation in 1,142 malaria mosquitoes

Alistair Miles
The Wellcome Trust Centre for Human Genetics

The Anopheles gambiae 1000 Genomes Project is sequencing the genomes of individual mosquitoes collected from natural populations across Africa. The goal of the project is to discover natural genetic variation, provide a data resource for the malaria vector research community, and study the evolutionary and demographic history of mosquito populations. I will describe results from phase 2 of the project, which comprises data on 1,142 mosquitoes sampled from 13 African countries. The phase 2 cohort includes several new mainland populations representing the incipient species An. gambiae and An. coluzzii, and introduces two new island populations, providing a rich resource for investigating speciation processes, patterns of mosquito migration, and genes under strong selective pressures. Within this cohort, we have discovered over 57 million single nucleotide polymorphisms (SNPs), corresponding to a variant allele at every 1.9 accessible bases of the genome on average. I will present evidence that high levels of standing genetic variation in mosquito populations, coupled with gene flow between populations, have driven a strong adaptive response to insecticides. The same factors could also impact on the efficacy of new vector control methods based on genetic modification, such as CRISPR/Cas9 gene drive. I will also present analyses on the ancient and recent demographic histories of the populations we have sequenced, and discuss the use of genome sequencing for prospective surveillance and monitoring of malaria vectors in Africa.

Divergent natural selection on eclosion time variation affects genome-wide patterns of geographic and host related differentiation for apple and hawthorn host races of *Rhagoletis pomonella*

Meredith M. Doellman[1], Scott P. Egan[1,2], Gregory J. Ragland[1,3], Peter J. Meyers[1], Glen R. Hood[1,2], Thomas H.Q. Powell[1,4,5], Daniel A. Hahn[4], Stewart H. Berlocher[6], Patrik Nosil[7], Jeffrey L. Feder[1]

[1] Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA; [2] Department of Biosciences, Rice University, Houston, TX 77005, USA; [3] Department of Integrative Biology, University of Colorado - Denver, Denver, CO, 80217, USA; [4] Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611; [5] Department of Biological Sciences, State University of New York – Binghamton, Binghamton, NY, 13902; [6] Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL, 61801; [7] Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK, S10 2TN

Taxa prone to ecological speciation may often possess large stores of standing variation, facilitating rapid divergence in response to ecological opportunity. One key factor contributing to standing variation may be the adaptive tracking of environmental variation by ancestral populations. Here, we characterize the geographic pattern of genomic differentiation for 10,241 SNPs in *Rhagoletis pomonella* (Diptera: Tephritidae) to infer the degree to which standing variation in adult eclosion time in the ancestral hawthorn-infesting (*Crataegus* spp.) host race of the fly contributed to its recent shift to earlier fruiting apple (*Malus domestica*). The genetic response in eclosion time was highly predictive of geographic variation across four latitudinally arrayed sites where apple and hawthorn flies co-occur in the Midwest U.S.A. The relationship was strongest for SNPs on chromosomes 1-3, especially those displaying high linkage disequilibrium within putative inversions. As predicted by host fruit phenology, alleles associated with later eclosion were present at higher frequencies at more southern sites. While this geographic pattern could explain why apple flies eclose earlier than hawthorn flies locally at the two more southern sites, it could not at the two northern sites. Further studies of gene-by-gene and gene-by-environment interactions are therefore required to fully resolve how standing geographic variation in the hawthorn race was selected to generate the earlier eclosing apple race at more northern sites and establish the generality of the results for *Rhagoletis* to other model systems of rapid ecological divergence.

Genomic, transcriptomic and epigenomic variations in response to host-plant in the agricultural pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Marion Orsucci, Sylvie Gimenez, Kiwoong Nam, Emmanuelle d'Alençon, Nicolas Nègre, The Fall Armyworm International Public Consortium

DGIMI; INRA; University of Montpellier, 34095 Montpellier, France

Spodoptera frugiperda, the Fall Armyworm (FAW) is an important agricultural pest in the American continent, causing damage to corn, sorghum and soybean. While the FAW caterpillars are considered polyphagous, a difference in diet preference has been described between two genetic variants: the Corn strain (sf-C) and the Rice strain (sf-R). Under the ecological speciation hypothesis, the host-plants should effect differently the overall fitness of FAW strains and the genes linked to plant adaptation should also be linked to reproductive isolation, thus creating reduction of gene flow between FAW populations living in sympatric areas. Our group is integrating ecological, genomic, transcriptomic and epigenetic data in order to understand if and how the mechanisms of plant adaptation promoted the divergence of *S. frugiperda* in two sympatric strains. Whole genome sequencing and assembly of *S. frugiperda*, followed by resequencing

of natural populations revealed a major split at the mitochondrial level between the two strains as well as islands of divergence in the nuclear genome (Gouin et al., Sci Reports 2016).

We performed controlled reciprocal transplant (RT) experiments to address the impact of plant diet on fitness of the sf-C and sf-R strains. We show that oviposition preferences differ between strains while larval development differences are caused by the plant diet. We also show that corn plants based diet have a positive impact on overall survival of sf-C. To understand the genetics basis of plant adaptation differences between strains, we analyzed by RNA-seq the gene expression of FAW larvae from the RT experiment. We show for each strain how the change of diet causes variation in gene expression of multigenic families of metabolic enzymes, as well as detoxification proteins. This response to host plants is different between the two strains and suggests that the metabolism pathways differ between them. We found consistent transcriptional differences between the strains, regardless of the rearing conditions. Some of these variations also occur in natural populations and involve mitochondrial genes, suggesting that energy production efficiency by the mitochondrion might be the main physiological difference between the strains. In this scenario, the separation of the strains could come from a different metabolic efficiency that is revealed when the moths are confronted to non-preferred plants.

We are currently investigating whether genetic variation at the level of regulatory elements, defined by chromatin landscapes, can explain the difference in genomic plasticity in plant adaptation between the two FAW strains.

FUNCTIONAL GENOMICS

Using functional genomics to explore arbovirus-host interactions

Sara Cherry
University of Pennsylvania

Our driving interest is to discover the spectrum of cellular factors at the virus-host interface and to elucidate the mechanisms by which pathogens subvert this cellular machinery while evading recognition. We use a combination of functional genomics coupled with cutting-edge molecular approaches to define both the players and mechanisms involved. We have performed a large number of genome-wide RNAi screens and these data have revealed fundamental insights into the plethora of pathways engaged to block infection. Furthermore, we have compared and contrasted host factor dependencies across diverse human arthropod-borne viruses exploring genes and pathways active in both insects and humans. We use the model organism Drosophila for its powerful genetic tools and focus on conserved genes that play parallel roles in both vectors and humans. Using this system we have identified complex mechanisms involved in viral RNA recognition and restriction. In addition, we have developed a system to explore enteric immunity to arboviruses using the Drosophila system where we have uncovered new roles for the microbiota in shaping antiviral defense. We will discuss our new findings on the pathways and players involved in arbovirus-host interactions.

Leveraging genomic approaches to study seasonal biology of Drosophilia suzukii

JoAnna Chiu
Department of Entomology and Nematology, University of California, Davis, CA

Drosophila suzukii is an invasive species that originated from Southeast Asia, and was first discovered in the continental U.S.A. (Watsonville, CA) in 2008. It has since rapidly spread to become an established pest of fruit crops all over the world. Commonly known as the Spotted Wing Drosophila (SWD), this vinegar fly has an enlarged, serrated ovipositor, allowing adult females to penetrate the skin of soft-skinned, ripening fruit and lay eggs inside, where the larvae feed and destroy the fruit. *D. suzukii* invasions have caused significant crop losses that amounts to millions of dollars annually, and disrupted previously successful integrated management programs on fruit crops. *D. suzukii* cold resistance studies predict that this species cannot overwinter in northern locations, e.g. Canada, but curiously they are established pests in these regions. Combining physiological investigations, RNA sequencing, and molecular characterization of candidate proteins, we present potential mechanisms by which *D. suzukii* can overwinter in northern latitudes. This work may contribute to more accurate population models that incorporate seasonal variation in physiological parameters, leading to development of better management strategies to combat this serious pest.

REDfly and *SCRMshaw*: powerful tools for insect regulatory genomics

Marc S. Halfon
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The number of sequenced insect genomes continues to grow at a rapid pace, but annotation of the regulatory component of these genomes lags significantly behind. Two resources developed by our group are helping to rectify this situation. *REDfly* (*R*egulatory *E*lement *D*atabase) is a comprehensive source of data on *Drosophila melanogaster cis*-regulatory sequences containing records for empirically validated *cis*-regulatory modules (CRMs, "enhancers") and transcription factor binding sites (TFBSs) reported in the published literature. *REDfly* currently covers approximately 700 publications and contains more than 22,000 records of reporter constructs regulating over 630 genes, including over 5800 "minimal" CRMs from transgenic in vivo reporter assays and over 10,000 from cell culture assays, as well as over 2000 TFBSs. A major feature of this resource is the detailed information available about the spatio-temporal patterns of gene expression regulated by each included CRM. Although currently restricted to data from *D. melanogaster*, the *REDfly* schema is easily expandable to include other insects, and plans are in place to begin including multiple insect species in the near future. CRM data from *REDfly* serve as input for *SCRMshaw*, a machine-learning computational approach for CRM discovery. *SCRMshaw* takes advantage of the enormous wealth of *D. melanogaster* CRM data to facilitate CRM discovery in not just *Drosophila* but in diverse insect species extending at least throughout the holometabola, including mosquitoes, beetles, bees, and wasps. Initial studies indicate that *SCRMshaw* is highly effective for cross-species CRM discovery with a high rate of return and a low false-positive prediction rate. Because the same training data are used by *SCRMshaw* to search all species, chances of discovering homologous (or functionally similar) CRMs for orthologous genes are strong, a decided advantage for evo-devo studies; several compelling examples have already been identified. We are developing approaches to combine *SCRMshaw* with comparative genomic data for closely-related species groups (e.g., Anopheline mosquitoes) and with empirical CRM-discovery methods such as open-chromatin profiling (FAIRE-seq, ATAC-seq). Both of these additions should enable substantial reductions in the already-low false positive rate to produce increased accuracy of prediction. Together, the combination of *REDfly* and *SCRMshaw* will allow for rapid and cost-efficient first-generation annotation of the regulatory genomes of a large fraction of currently-sequenced insects. *REDfly* is freely accessible at http://redfly.ccr.buffalo.edu and can be followed on Twitter at @REDfly_database. *SCRMshaw* software can be downloaded from http://veda.cs.uiuc.edu/SCRMshaw. This work was supported by grants from the NSF, NIH, and USDA.

Regulation of alternative splicing during the nurse-forager transition in *Apis mellifera*

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Phenotypic plasticity allows individuals to, for example, adapt their physiological responses to their environment, as well as to the stage of their life cycle. Within a honeybee colony, worker bees start their adult life as nurses involved with in-hive tasks. As such, nurse bees only need to fly limited distances and for short amounts of time. Some of the nurse bees transition later in life (around 21 days) to foragers whose job is to collect water and nectar, and as such foragers need to be excellent flyers. The nurse-forager transition event is a model system for understanding the molecular implication of phenotypic plasticity. This event

necessitates major changes in the bee‘s behavior, but also in the efficiency and power generated by the flight muscle system (IFM). RNA sequencing analysis of flight muscle tissues isolated from nurse and foragers at different ages reveal differential gene expression of genes involved in several biological processes, including stress response, immunity, and protein synthesis. Proteins involved in muscle sarcomere structure undergo shift between isoforms during the transition. The generation of these alternative splice variants depends on the activity of regulatory splicing factors, such as muscleblind, Aret, How, and others. For example Troponin T is expressed as two isoforms, one specific to the IFM. The ratio between the two isoforms changes during the nurse-forager transition and can be correlated with the differential expression of the Muscleblind splicing factors. Manual annotation and expression analysis of several muscle protein isoforms and regulatory splicing factors across behaviors and age will be presented.

VECTOR GENOMICS

Comparative transcriptomics across seven members of the genus Aedes

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Aedes aegypti is the primary vector of arboviruses worldwide, responsible for the majority of Zika, dengue and chikungunya virus transmission. Despite its central role in pathogen transmission, only one other member of the genus has been sequenced, making it difficult to determine evolutionary trends and patterns among genes in this mosquito. In particular, work in the model organism Drosophila has demonstrated that critical components of the anti-viral RNAi pathway are among fastest evolving genes in the fly genome, suggesting an active arms race with viral pathogens seeking to suppress or escape from this defense system. To determine if this is also the case for important disease vectors, and to identify other immune genes that may be rapidly evolving or under selection, we sequenced and assembled transcriptomes for the mosquitoes *Aedes hensilli, Aedes vexans, Aedes triseriatus, Aedes japonicus* and *Aedes canadensis*. Along with current gene models for both *Ae. aegypti* and *Ae. albopictus*, we established orthologous clusters, removed those containing ambiguous relationships or in-paralogs using orthomcl, and performed a multiple sequence alignment for each remaining cluster. Finally, about 9000 passing cluster alignments were analyzed via codeml for dN/dS ratios and determination of positively selected sites. Contrary to what was found with Drosophila, RNAi components such as dcr2 and r2d2 were found to be evolving around the genomic average, and hence appear to be under much more severe purifying selection in Aedes mosquitoes. However, many other immune gene families were enriched for fast evolving genes, such as AMPs, Clip-domain serine proteases and Serpins. These transcriptome resources described herein will likely be valuable for analyzing the evolutionary history of other important physiological pathways across the genus Aedes.

RNAi interference: receptive and unresponsive spaces and places in the body of a mosquito

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RNAi is a powerful antiviral innate immune response, and a powerful tool for reverse genetics. Despite our advances in fine-tuning RNAi to develop virus-resistant mosquitoes and many hundreds of studies in which RNAi has been used to assess gene function, we do not have a clear understanding of best practices to maximize successful implementation of this technique. Most of our labs likely have anecdotal and published evidence of widely variable success in the use of RNAi. The source of this variability could be in the design of the RNAi trigger, in the uptake and processing, or degradation, of RNAi triggers in specific tissues of interest, or in RNAi trigger dissemination. Further, this could differ between species, or even in an individual according to physiological status. To begin to understand this variability, we undertook a meta-analysis of the literature in which RNAi has been employed in mosquitoes to identify determinants of RNAi success, microscopic studies of RNAi trigger uptake in multiple tissues and species, and molecular and phenotypic analysis of RNAi trigger processing.

Dynamics Between Dehydration, Carbohydrate Metabolism, and Blood Feeding in Mosquitoes Revealed by Combined Transcriptomic and Metabolomic Analyses

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Mosquitoes are a major contributor in the transmission of arthropod-borne diseases. Despite extensive focus on the relationship between mosquitoes, environment, and disease transmission, the role of dehydration on mosquito biology is vastly understudied when compared to thermal effects. In this study, *Culex pipiens* were exposed to short bouts of dehydration and phenotype changes were analyzed with metabolomics, RNA-seq, and behavioral/physiological monitoring. To differentiate between only exposure to desiccation conditions versus actual water loss, groups were subjected to dehydration with and without access to free water or were permitted a rehydration opportunity following water loss. The groups with access to free water or following rehydration displayed no behavioral changes, while activity and host landing along with blood feeding were increased in dehydrated *C. pipiens.* Complementary results were noted in two other mosquitoes, *Aedes aegypti* and *Anopheles quadrimaculatus*. Metabolomics and RNA-seq revealed altered trehalose to glucose conversion during dehydration, but we noted negligible alterations in major nutrient reserve levels, suggesting that this is not a starvation response. Targeting *trehalase* with RNA interference suppressed trehalose breakdown and decreased dehydration-associated phenotypes associated with increased blood feeding. These results suggest dehydration stress prompt mosquitoes to seek a bloodmeal as a rehydration mechanism and this shift will likely alter disease transmission.

Structural variant detection by read-cloud sequencing in *Aedes aegypti*

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The recent re-emergence of Zika virus has focused attention on the arbovirus vector *Aedes aegypti*, a mosquito which is also transmits dengue virus, yellow fever and chikungunya. Yet compared to the better-studied *Anopheles* mosquitoes that transmit malaria, little is currently known about the population genomics and global population structure of *Aedes aegypti*. A large and repetitive genome, high diversity, and potentially extensive structural variation combine to hamper genomic analyses, and until this year had prevented even the full assembly of the genome. Structural variants have proven to be key features for studying dipteran population structure. Across the full range of the order, large chromosomal inversions exist both as clearly identifiable markers distinguishing sub-populations, as well as potential sites of divergence that could underlie speciation. Although the intractable genome of *Aedes* has prevented any such survey being carried out in the past, characterising structural variation will be an important asset for mapping insecticide resistance markers, examining population structure, or identifying suitable targets for gene-drive systems, among other approaches. Building on the work performed by the Aedes Genome Working Group, we have performed the first large-scale investigation of structural variation in *Aedes aegypti*. Using linked-read (10X) technology and a variety of algorithmic approaches we have identified segregating inversions in a world-wide sample set along with a large number of duplications and deletions.

By generating an initial catalogue of structural variants in this species we hope that this work will provide the foundation to subsequent studies of *Aedes* population structure and the potential for disease transmission by this vector.

METAGENOMICS

Microbiota integration in tsetse biology

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Tsetse flies (Diptera: Glossinidae) have high medical and economic significance as the obligate vector of African trypanosomes. Adult flies have a unique feeding ecology of only vertebrate blood that is enabled through essential nutrient provisioning by the digestive tract microbiota. This simple microbiota is primarily made up of two Gammaproteobacteria; *Wigglesworthia* and *Sodalis spp.*. These bacteria have very different co-evolutionary histories with tsetse and, consequently, contributory roles towards host biology. The sequencing and annotation of the *Wigglesworthia* and *Sodalis* genomes have enabled several functional genomic studies revealing multiple roles in tsetse nutritional biology, immunology and vector competency. These symbiont roles were primarily characterized within colony flies, but recent Illumina-based transcriptomics of field flies strongly corroborate these results. The contributions of the microbiota towards various aspects of tsetse fly biology will be discussed. Eliminating essential microbiota members, or inhibiting their benefits to vector fitness, may give rise to novel tsetse-specific suppression methods.

Lipidomic and Proteomic Analyses of Flavivirus – Vector Interactions

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Flaviviruses, which include dengue, Zika, and West Nile viruses, are enveloped positive strand RNA viruses that are transmitted primarily by mosquitoes or ticks. Upon infection, the viruses in this genus induce substantial rearrangements in sub-cellular structure to facilitate the replication of their genome RNA and the encapsidation of that genome into a newly assembled virus particle. We have taken a multi-track approach to understand how the virus influences both mammalian and insect host cells in this process. A focus on structural biology and proteomics and metabolomics has demonstrated that these viruses induce the synthesis of specific lipids that support the virus life cycle. In dengue virus infection ceramide and sphingolipids are significantly increased and support virus replication. Inhibitors of fatty acid metabolism have detrimental impacts on virus replication. Although similar, infection of human and mosquito cells also have unique differences that may be exploited for future antiviral therapeutics.

Mapping QTLs in *Culex quinquefasciatus* that control the density of bacterial symbiont *Wolbachia pipientis*

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The unique characteristics of *Wolbachia* are being exploited to develop *Wolbachia* infection of vector mosquitoes as an approach for interrupting the transmission cycle of viral disease pathogens. This approach hinges on the ability of *Wolbachia* infection to increase host resistance to viral pathogens, determined, in part, by the density of *Wolbachia* in host tissues. Little is known, however, about how *Wolbachia* density is regulated in native or heterologous hosts. We measured the broad-sense heritability of *Wolbachia* density between families in field populations of the mosquito *Culex pipiens*, and found that densities in ovary and non-gonadal tissues of females in the same family are not correlated, suggesting that *Wolbachia* density is determined by distinct mechanisms in the two tissues. Using introgression analysis between two different strains of the closely-related species *Culex quinquefasciatus*, we found that *Wolbachia* densities in ovary tissues are determined by cytoplasmic genotype, while densities in non-gonadal tissues are determined by both cytoplasmic and nuclear genotypes and their epistatic interactions. Quantitative-trait locus mapping using a new high-density SNP-based genetic map of the *Cx. quinquefasciatus* genome identified two major-effect quantitative-trait loci explaining a combined 23% of variance in *Wolbachia* density specifically in non-gonadal tissues. A better understanding of how *Wolbachia* density is regulated will provide insights into how *Wolbachia* density can vary spatiotemporally in mosquito populations, leading to changes in *Wolbachia*-mediated phenotypes such as viral pathogen resistance.

Discovery of Known and Novel Viral Genomes in Soybean Aphid by Deep Sequencing

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The soybean aphid (Aphis glycines) is one of the most economically important pest insects of soybean. Viruses of the soybean aphid have not been studied. To explore the soybean aphid virome (the collection of viruses found in and on the soybean aphid) and to seek viruses as potential biological control agents, we employed Illumina sequencing. Genomes isolated from viruses in soybean aphids collected at four sites revealed many viruses, and six complete or nearly complete genomes were assembled. Most abundant were the picornavirus-like dicistroviruses Aphid lethal paralysis virus and Rhopalosiphum padi virus. We also sequenced the genome of a new dicistrovirus, Big Sioux River virus, fragments of which had been found previously in honey bee. Genome sequences that represent two entirely new virus families were obtained. These include an abundant tetravirus-like virus and a virus distantly related to cileviruses of plants and negeviruses of insects. Surprisingly, Cotton leafroll dwarf virus, a member of the genus Polerovirus, was found in aphids from China. This virus had not been reported previously in China or in soybean. This study provides a peek into the rapidly expanding, largely unexplored world of insect viromes that will provide valuable knowledge for future understanding of plant–virus–vector interactions. The methods described here can be used to sequence the viromes of any insect or other small arthropod.

SENSORY GENOMICS

Functional analysis of insect sweet taste receptors

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The ability to identify nutritious food sources for consumption and toxic substances to avoid is important for survival. Insects possess members of a highly diverse family of Gustatory receptors (Gr), which are expressed in gustatory receptor neurons (GRNs) and mediate recognition of sweet and bitter compounds. In *Drosophila, e*ight Gr receptors belonging to an evolutionarily conserved clade within the Gr family, are expressed in sweet taste neurons and required for detection of sugars and other sweet compounds. To elucidate response properties of individual sweet Gr receptors, we developed an *in vivo* functional expression system. We expressed each of these Grs singly in the ab1C carbon dioxide-sensing olfactory neuron and tested whether expression was sufficient to confer sensitivity to any sweet compounds. We found that each sweet Gr responded to unique but overlapping subsets of sweet sugars. We also tested Gr43a, an internal fructose receptor and its *Anopheles gambiae* ortholog, AgGr25, and found that both receptors confer sensitivity to fructose and other sugars when expressed in the *Drosophila* ab1C neuron. Thus, all sugar receptors appear to be directly involved in detection of sweet compounds. We and others found that sweet taste neurons are also inhibited by aversive tastants. Using the ab1C ectopic expression system, we tested individually expressed sweet taste receptors against a diverse panel of bitter compounds and found that sweet Grs could be directly inhibited by bitter tastants. Different Grs exhibited different sensitivities for different bitter compounds, and each Gr showed a distinct inhibitory response profile. Inhibition by bitter tastants appears to be a property of the sweet receptor clade – neither Gr43a, nor the carbon dioxide receptor Gr21a/Gr63a were inhibited by any bitter tastants. Analysis of *Anopheles gambiae* sweet receptors by functional expression in the fly ab1C neuron suggests that cross-modality interactions of sweet taste receptors with bitter compounds are conserved across millions of years of evolution

A Screen for Chemosensory Gal4 Drivers that function in Multiple Dipteran Insects

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To study and eventually manipulate insect behaviors, researchers require tools to identify neurons required for the behavior, determine how activity of the neurons influences the behavior, and explore how the neurons connect to other participating neurons. While such tools are increasingly available for analysis of genetic model organisms such as Drosophila melanogaster, we presently lack sophisticated genetic tools for analysis of the neurobiological basis of behaviors in non-model insects. The binary expression system Gal4-UAS, in which temporal and tissue-specific expression of the Gal4 transcriptional activator drives expression of a UAS responder, is routinely utilized for neurobiological studies in D. melanogaster. Although the Gal4-UAS system has been introduced in other insects, including several mosquito species, very few Gal4 and UAS strains have been developed outside of Drosophila. To address this, we recently completed a FAIRE-seq open chromatin profiling study in Aedes aegypti, the principle mosquito vector for Zika, yellow fever, chikungunya, and dengue viruses. This study resulted in identification of >121,000 putative cis-regulatory elements in the A. aegypti genome. Subsequently, we conducted a reporter screen to identify regulatory elements with a strong likelihood of functioning in

chemosensory tissues, particularly the olfactory system, of multiple dipteran insects. The screen, which was performed in D. melanogaster using FAIRE-seq identified putative regulatory elements from A. aegypti, uncovered many regulatory elements, a subset of which drove gene expression in chemosensory systems. Several of the elements that drive olfactory-specific gene expression were cloned into a Gal4 transgenic vector that is compatible with multiple modes of transformation of A. aegypti and other insects. An A. aegypti Gal4 driver that promotes gene expression in olfactory receptor neurons was identified and characterized. Several additional A. aegypti olfactory driver lines, including one that generated sex-specific neural gene expression in Drosophila reporter assays, are presently being generated. The transgenic constructs developed for these assays can be used directly to transform additional insect species. Future studies will focus on generation of additional Gal4 drivers, as well as UAS responder lines, that will allow us to study and manipulate insect chemosensory systems.

Evolutionary History of Chemosensory-Related Gene Families across the Arthropoda, with special focus on the Pancrustacea

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Chemosensory-related gene (CRG) families have been studied extensively in insects, but their evolutionary history across the Arthropoda had remained relatively unexplored. Here, we address current hypotheses and prior conclusions on CRG family evolution using a more comprehensive dataset. In particular, odorant receptors (ORs) were hypothesized to have proliferated during terrestrial colonization by insects (hexapods), but their association with other pancrustacean clades and with independent terrestrial colonizations in other arthropod subphyla have been unclear. We also examine hypotheses on which arthropod CRG family is most ancient. Thus, we reconstructed phylogenies of CRGs, including those from new arthropod genomes and transcriptomes, and mapped CRG gains and losses across arthropod lineages. Our analysis was strengthened by including crustaceans, especially copepods, which reside outside the hexapod/branchiopod clade within the subphylum Pancrustacea. We generated the first high-resolution genome sequence of the copepod Eurytemora affinis and annotated its CRGs. We found ORs and odorant binding proteins (OBPs) present only in hexapods (insects) and absent from all other arthropod lineages, indicating that they are not universal adaptations to land. Gustatory receptors (GRs) likely represent the oldest chemosensory receptors among CRGs, dating back to the Placozoa. We also clarified and confirmed the evolutionary history of antennal ionotropic receptors (IRs) across the Arthropoda. All antennal IRs in E. affinis were expressed more highly in males than in females, suggestive of an association with male mate-recognition behavior. This study is the most comprehensive comparative analysis to date of CRG family evolution across the largest and most speciose metazoan phylum Arthropoda.

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Evolution of chemical communication in a pest fly, Drosophila suzukii

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Insects interpret the rich chemical landscape around them through a combination of physiological and behavioral adaptations. Since animal senses are biological features that have been shaped by natural selection to promote adaptive behavior, a variety of exciting patterns are apparent in what they sense and how. Drosophila display robust olfactory driven behaviors. A distinct yet limited range of volatile organic compounds are used as major cues for finding suitable substrates to feed or oviposit, and find mates. I will present our recent findings that demonstrate how a pestiferous and invasive fly, *D. suzukii* employs olfaction as a key sensory modality in finding hosts and mates. In addition, data from the phylogenetically close and distant relatives reveals a strong co-evolutionary pattern in the chemical signatures and their molecular correlates for reception.

GEORGE B. CRAIG, JR. MEMORIAL LECTURE

Public Health Entomology and Vector Control in the Greater Mekong Subregion

Michael Macdonald, ScD

The emergence of artemisinin-resistant falciparum malaria in the Greater Mekong Subregion (GMS) has brought increased investment and response for control efforts. Initially, this was the launch in 2011 of a 'containment' strategy for what was feared to be the spread of resistant parasites to other regions; and then in 2015, the endorsement of an elimination strategy for the six countries of the subregion to be malaria-free by 2030. Entomology and Vector Control in the GMS must adapt to the changing context. This includes, first, shifting entomology strategies from control to elimination through risk area stratification and "Hazard Mapping", expanding and decentralizing sampling and increased use of GIS and Remote Sensing, and improved capacities for foci investigation and elimination. Second, adapting vector control to fit the context, especially in developing new tools and strategies for personal protection for outdoor and residual transmission. These efforts to develop new tools in the Mekong are linked to a broader initiative though RBM for Vector Control in Humanitarian Emergencies, where many of the same treated materials or repellents could be used in for displaced persons and refugees where traditional mosquito nets and Indoor Residual Spraying are not practical. Third, and most fundamental, is capacity with ongoing investment for infrastructure: insectaries, reference labs and GIS platforms; and for human resources: posts, career opportunities and training, networks and university linkages.

NOTES

Poster Presentations

1. Molecular mechanisms underlying pregnancy in the live-bearing cockroach, *Diploptera punctate*

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Viviparous reproduction is characterized by retention of developing offspring within the mother's reproductive tract during gestation, culminating in live birth. In some cases, a mother will provide nutrition beyond that present in the yolk; this is known as matrotrophic viviparity. While this phenomenon is best associated with mammals, it is observed in insects such as the viviparous cockroach, *Diploptera punctata*. Female *D. punctata* carry their embryos in the brood sac, a reproductive organ that acts as both a uterus and placenta. During pregnancy, mothers provide a nutritive secretion to the intrauterine developing progeny. While the basic physiology of *D. punctata* reproduction has been characterized, little is known about the molecular mechanisms underlying this process. This study integrates RNA-seq, RNA interference, and other assays to characterize molecular changes associated with *D. punctata* reproduction. Additionally, we provide the most complete gene set to date for this species. A comparison of four stages of the female reproductive cycle revealed unique transcriptional profiles as pregnancy progresses. Differentially expressed transcripts of interest include the previously identified family of milk proteins, transcripts associated with juvenile hormone metabolism, and other reproduction-associated transcription factors. RNA interference experiments reveal potential impacts of juvenile hormone breakdown in maintaining pregnancy in *D. punctata*.

2. RNA-seq and metabolomic analyses reveal molecular mechanisms underpinning survival of ticks (Ixodidae) following environmental and physiological stress

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Ticks are obligate blood feeders, but spend the majority of their lives off hosts where they must contend with a multitude of stresses that can influence distribution and population dynamics. Ticks must tolerate extended periods of thermal stress, desiccation, and lack of food; however, little is known about the mechanisms underlying survival of ticks under these stresses. To uncover specific aspects associated with stress survival in ticks, we examined the transcriptomic responses of several hard ticks (Ixodidae) in relation to thermal stress, dehydration, and starvation. RNA sequencing was used to analyze transcriptional changes following stress exposure and revealed 500 to ~3,000 differentially expressed genes with the enrichment of stress-response and proteolysis pathways as well as shifts in expression of metabolic-related genes. Up- and down-regulated pathways were compared among stresses and species to identify common stress response genes. Follow up analyses found the accumulation of several metabolites, including amino acids, suggesting these molecules may act as cryo- and/or osmoprotectants. Finally, analysis of tick energetics demonstrated that lipid and protein resources are critical for energy during extended starvation and sufficient stores of these reserves are critical to stress tolerance. Overall, our results identified specific molecules and pathways that contribute to tick stress tolerance and lay the groundwork for further studies on stress for these important disease vectors.

3. Combined genomic and transcriptomic analyses of the stable fly reveal mechanisms underlying reproduction, host interactions and pest control

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The stable fly, *Stomoxys calcitrans*, is a major blood feeding livestock pest that has near worldwide distribution with an annual cost of over $2.2 million in the United States due to control measures and product loss. Few genetic and molecular tools are available to develop novel control protocols for stable flies. This study utilized the combination of genome sequencing and RNA-seq analyses targeting multiple developmental stages and tissues to examine stable fly biology. Specifically, we examine novel genes associated with the saliva, sex- and reproductive-associated gene, aspects related to host location, and pesticide resistance and detoxification. The combined sequencing, assembly, and curation of the stable fly genome followed by RNA-seq provide insights necessary to understand the biology of this important pest. These resources and knowledge will provide the groundwork for studies that enhance integrated pest management strategies for the suppression of stable fly infestations. The close relationship between other blood feeding *Glossina* and non-blood feeding flies (medflies, *Drosophila*, house flies) will broaden our understanding of aspects underlying the evolution of blood feeding among higher flies.

4. Innate Parasitoid Resistance in the Potato Aphid, *Macrosiphum euphorbiae*

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Macrosiphium euphorbiae is an economically important pest, vectoring over 50 plant viruses throughout major crops such as tomato, strawberry, raspberry and potato. Integrated pest management (IPM) systems for *M. euphorbiae* could be developed to make use of natural enemies of the aphid, such as parasitoid wasps. Previous research has identified eight genotypes within this aphid species, one of which exhibits innate resistance to the generalist parasitoid *Aphidius ervi*; this finding contrasts with some other aphid species, where parasitism-resistance can be conferred by facultative bacterial endosymbionts. Through genomic studies, we aim to clarify the mechanism of aphid-encoded innate resistance. Aphids were sampled from commercial fields and garden sites in Summer 2016, with aphid genotype and parasitoid susceptibility assessed. Seven lines of *M. euphorbiae* showing low parasitoid susceptibility were sequenced on Illumina and one line using 10x Chromium platform. Analysis of these genomes was conducted to check for the presence of cryptic endosymbiont that might confer resistance. However, this screen did not uncover any such cryptic symbionts, providing additional evidence that this parasitoid resistance the result of innate immunity. Further analysis involves looking for genomic regions under selection and using RNAseq to discover genes involved in the process of resistance.

5. The sequence of a male-specific genome region containing the sex determination switch in *Aedes aegypti*

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Aedes aegypti is the principal vector of several important arboviruses. Among the methods of vector control to limit transmission of disease are genetic strategies that involve the release of sterile or genetically modified non-biting males, which has generated interest in manipulating mosquito sex ratios. Sex determination in *Ae. aegypti* is controlled by a non-recombining Y chromosome-like region on autosome 1 called the M locus, yet characterisation of this locus has been thwarted by the repetitive nature of the genome. In 2015, an M locus gene named *Nix* was identified that exhibits the qualities of a sex determination switch. With the use of a whole-genome BAC library, we amplified and sequenced a ~200kb region containing this male-determining gene. In this study, we show that *Nix* is comprised of

two exons separated by a 99kb intron, making it an unusually large gene. The intron sequence is highly repetitive, and we speculate that the lack of recombination at the M locus has allowed the expansion of repeats in a manner characteristic of a sex-limited chromosome, in accordance with proposed models of sex chromosome evolution in insects.

6. Comparative phylogenetics and genome evolution of the global agricultural pest, Colorado potato beetle

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The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say, is a significant agricultural pest on cultivated *Solanum* crops such as potato, tomato, and pepper (Grapputo et al., 2005). The *Leptinotarsa* genus comprises 41 known species distributed from North to South America, with the center of diversity located in Mexico (Jacques 1988). To date, there is no phylogeny on this genus. In order to elucidate the genomic changes associated with the development of CPB as a pest, we compare whole genomes of ten closely related *Leptinotarsa* species from North America and Mexico. A species tree was constructed using these whole genome sequences to provide a tool for further investigation. Using this phylogeny as a backbone, we examine gene family expansion/contraction events and rapid protein evolution tied to the emergence of this successful agricultural pest.

7. Genomic, transcriptomic and epigenomic variations in response to host-plant in the agricultural pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Spodoptera frugiperda, the Fall Armyworm (FAW) is an important agricultural pest in the American continent, causing damage to corn, sorghum and soybean. While the FAW caterpillars are considered polyphagous, a difference in diet preference has been described between two genetic variants: the Corn strain (sf-C) and the Rice strain (sf-R). Under the ecological speciation hypothesis, the host-plants should affect differently the overall fitness of FAW strains and the genes linked to plant adaptation should also be linked to reproductive isolation, thus creating reduction of gene flow between FAW populations living in sympatric areas. Our group is integrating ecological, genomic, transcriptomic and epigenetic data in order to understand if and how the mechanisms of plant adaptation promoted the divergence of *S. frugiperda* in two sympatric strains. Whole genome sequencing and assembly of *S. frugiperda*, followed by resequencing of natural populations revealed a major split at the mitochondrial level between the two strains as well as islands of divergence in the nuclear genome (Gouin et al., Sci Reports 2016).

We performed controlled reciprocal transplant (RT) experiments to address the impact of plant diet on fitness of the sf-C and sf-R strains. We show that oviposition preferences differ between strains while larval development differences are caused by the plant diet. We also show that corn plants based diet have a positive impact on overall survival of sf-C. To understand the genetics basis of plant adaptation differences between strains, we analyzed by RNA-seq the gene expression of FAW larvae from the RT experiment. We show for each strain how the change of diet causes variation in gene expression of multigenic families of metabolic enzymes, as well as detoxification proteins. This response to host plants is different between the two strains and suggests that the metabolism pathways differ between them. We found consistent transcriptional differences between the strains, regardless of the rearing conditions. Some of these variations also occur in natural populations and involve mitochondrial genes, suggesting that energy production efficiency by the mitochondrion might be the main physiological difference between the strains. In this scenario, the separation of the strains could come from a different metabolic efficiency that is revealed when the moths are confronted to non-preferred plants.

We are currently investigating whether genetic variation at the level of regulatory elements, defined by chromatin landscapes, can explain the difference in genomic plasticity in plant adaptation between the two FAW strains.

8. Analyzing insertion-deletion variants in *Anopheles coluzzii* and *An. gambiae*

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Population genomics studies frequently rely on single nucleotide polymorphisms (SNPs) while ignoring longer variants, including insertion-deletion variants (indels). Indels tend to be harder to identify and validate than SNPs, and much of the population genomics analysis ecosystem has grown up around SNPs. In some organisms, indels easily detectable from short-read data are less abundant than SNPs, leading to the misconception that they are unimportant.

However, data from multiple species increasingly emphasize the importance of indels. In *Plasmodium falciparum*, indels are more numerous than SNPs in the core genome, and in the human genome, indels affect more total bases than do SNPs. Indels in the human genome have also been associated with expression-level variation; coding region indels, which have the potential to be particularly disruptive, have been associated with disease phenotypes in mouse models.

The *Anopheles gambiae* complex is one of many systems in which indels are acutely understudied. To address this, we called indels in publicly-available short reads, generated by the MalariaGen Ag1000G project from 22 *An. coluzzii*, *An. gambiae*, and hybrid samples resulting from a controlled cross between the two species. Using methods developed in *Plasmodium*, we validated indels by identifying putative violations of expected Mendelian segregation, and used variants with and without these violations to train the Genome Analysis Toolkit's variant recalibration machine learning method. We identified six variant annotations with good predictive power of whether a variant included a violation, and used these annotations to produce a set of high-confidence indels in coding regions. These indels showed characteristics consistent with expectations for coding regions, including strong enrichment for allele length changes in multiples of three. Finally, we genotyped wild-caught *An. gambiae* at these indels, using our high-confidence set to avoid the challenges of validating *de novo* indel calls in samples for which no Mendelian segregation data is available. We found that more than 90% of our high-confidence indels identified in the cross were also present in the wild-caught samples.

9. Genome sequence of the two-pronged bristletail *Campodea augens* (Diplura: Campodeoidea)

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In the last few years, genome sequencing projects have greatly expanded the sampling of Hexapoda species but genomic resources within Entognatha are scarce, representing a limitation for the study of Hexapoda evolution. In the framework of the *i*5k initiative, we embarked on the genome sequencing of species from Entognatha as well as from early-divergent insect clades. In this study, we present the draft genome sequence of *Campodea augens*, a species belonging to Diplura (two-pronged bristletails), a group of primarily wingless and blind soil-dwelling arthropods. Around 1,000 dipluran species have been described so far but due to their limited economic importance and subterranean lifestyle little information is available on their ecology and genetics. Nevertheless, Diplura is a key taxon to understand early splits in hexapod evolution.

The sequencing of *C. augens* genome was performed with a HiSeq 2000 Illumina platform employing 4 paired-end short insert libraries (2 X 350bp, 2 X 550 bp insert sizes) and 4 mate pair libraries (3, 6, 9 and 12 Kbp insert sizes). After testing several assemblers, the final assembly was performed using Platanus. Contigs were scaffolded with SSPACE v3.0 and gaps resolved using GapCloser v1.12. Additional tools namely Redundans and AGOUTI were employed for improving assembly quality. The final assembly spans around 1.2 Gbp, which corresponds to the

genome size estimated by flow cytometry. The contig N50 value is around 32Kbp and scaffold N50 around 300 Kbp. The assembly contains 98% (91% as complete genes, 7% as fragmented) of 1066 single-copy genes conserved in arthropods as assessed by BUSCO v2.1. The characterization of gene families and molecular pathways involved in perception, detoxification and immunity might reveal interesting features of this enigmatic species. The *C. augens* genome improves the sampling density within Diplura and offers new opportunities for evolutionary, ecological, and behavioral studies.

10. **What is a crustacean? Comparative genome analysis of crustaceans and insects**

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Arthropods comprise the largest and most speciose phylum on earth. However, comparative genomics within the subphylum Pancrustacea has focused predominantly on the insect clade (Hexopoda) and the clade that includes insects and the Branchiopoda (e.g. *Daphnia*). Thus, it is unclear which genomic properties characterize insects, relative to crustaceans as a whole. With the advent of high throughput sequencing technology and the i5k genome project, we are now able to analyze crustacean genomes outside of the Hexopoda/Branchiopda clade (e.g. insects and *Daphnia*). We performed comparative genome analysis of 11 Pancrustacean genomes both within and outside of the insect/branchiopod clade, along with a Myriapod outgroup genome. We found an interesting pattern of AT richness and gene families that are unique to insects. We also annotated previously unknown crustacean gene families and elucidated evolutionary patterns of several gene families that are related to environmental adaptation. Our results are a first step toward revealing the distinguishing features of insect and crustacean genomes, which are critical for yielding insights into human health, agriculture, and the environment.

11. De novo **sequencing of Argentine stem weevil and its parasitoid wasp biocontrol agent**

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The Argentine stem weevil (ASW), *Listronotus bonariensis* (Coleoptera: Curculionidae), is an exotic pasture pest in New Zealand. It was first detected in the 1920s, and by the 1980s was causing hundreds of millions of dollars' damage to pasture per year. In the early 1990s, a parasitoid wasp from the host range of ASW, *Microctonus hyperodae* (Hymenoptera: Braconidae), was introduced as a biocontrol agent. Attack rates were initially around 80%, providing effective control, but have subsequently declined to around 20%. Along with the agricultural importance of this problem, measuring genetic variation in both the sexual host and the asexual parasitoid is an exciting opportunity to investigate the evolution of host resistance in a biocontrol system.

We are working on genome and transcriptome assemblies for both species. This is a challenging project, because ASW is difficult to culture in the laboratory for inbreeding and field populations are heterogeneous, meaning that we had to limit the number of individuals used for sequencing. Both insects are small, limiting the amount of DNA that we can extract per individual, and our sequencing results indicate GC content around 30%, ruling out PCR-based genome amplification. We are also using genotyping-by-sequencing to survey ASW field populations and 16S re-sequencing to characterize endosymbiont diversity, and developing a high throughput resequencing assay to detect parasitoid DNA inside the host. These results are guiding our efforts to determine the mechanism of resistance using functional experiments including RNAi and genome editing.

12. **Assembling the genome of the fungus fly, *Sciara coprophila*, using a mixture of single-molecule technologies**

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Biological studies of the fungus fly, *Sciara coprophila*, began in the early 1900s and gave rise to an early example of an organism that broke the rule of DNA constancy. In Sciara, specific-loci are amplified, entire chromosomes are eliminated, and a single nucleus can contain over 8,000 copies of the genome. The phenomenon of imprinting was also discovered first in Sciara when it was observed that it was the paternal chromosomes that are specifically targeted for chromosome elimination in normal development. Moreover, Sciara females have either only male or only female offspring and since matings can be controlled to produce either, this makes Sciara a useful model system for sex-specific early development studies. However, studies into these and other interesting biological features of the fungus fly have been hampered by the lack of a genome sequence. We approached assembling the genome with multiple technologies. Using short read, paired-end Illumina data, we generated 40 different assemblies from seven popular assemblers. After evaluating the assemblies with several metrics, we were able to identify the best assemblies. Nonetheless, all short read assemblies were highly fragmented even after filtering for contamination and re-assembling. In contrast, we were able to produce highly contiguous assemblies with multi-megabase contigs using long reads from single molecule sequencing technologies including PacBio and the Oxford Nanopore MinION. As part of the MinION access program (MAP), we developed protocols to obtain very long reads, some of which exceed 100 kb. Not only do these ultra-long reads map to PacBio-only assemblies, they were very useful in assembling the Sciara genome. We generated 50 assemblies from 6 long read assemblers. Through extensive evaluation using dozens of reference-free metrics, we identified the best assemblies. Importantly, the assemblies generated from the combination of PacBio and Oxford Nanopore datasets typically outperformed PacBio-only assemblies in the majority of metrics. Optical maps from BioNano genomics were used to scaffold a subset of the best assemblies. RNA-seq datasets from a combination of embryos, larvae, pupae, and adult flies from both sexes were used to facilitate annotation of the final genome sequence. Finally, both PacBio and Oxford Nanopore data gave us the opportunity to explore DNA modifications in the Sciara genome sequence to potentially begin to unravel the mechanism of imprinting. Our work highlights the strengths of using single-molecule data to both assemble the genome of any arthropod and to begin an exploration of DNA modifications therein.

13. **Biocuration and improvement of the *Diaphorina citri* draft genome assembly with long reads, optical maps and long-range scaffolding**

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The Asian citrus psyllid (*Diaphorina citri* Kuwayama) is the insect vector of the bacterium *Candidatus* Liberibacter asiaticus (CLas), the causal agent for the citrus greening or Huanglongbing disease which threatens citrus industry worldwide. This vector is the primary target of approaches to stop the spread of the pathogen. Accurate structural and functional annotation of the psyllid's gene models and understanding its interactions with the pathogenic bacterium, CLas, is required for precise targeting using molecular methods. The draft genome was annotated with automated pipelines. Knowledge transfer from well-curated reference genomes like *Drosophila* to a newly sequenced insect is challenging due to the diversity and complexity among all insect genomes. We opted for manual curation of gene families that have key functional roles in *D. citri* biology and pathology. The community effort resulted in Official Gene Set v1.0 (aSaha 2017) with more than 500 manually curated gene models across developmental, RNAi regulatory, and immune-related pathways (bSaha 2017). Curators included undergraduate and graduate students

from multiple institutions as well as expert annotators from the i5k community. More information about our annotation initiative is available here https://citrusgreening.org/annotation/index.

Single copy marker analysis using BUSCO (Simão et al., 2015) of the current genome shows a significant proportion of 3,350 single-copy markers that are conserved in Hemipterans to be missing (25%) with only 74% present in full-length copies. The manual genome annotation also identified a number of mis-assemblies and missing genes in the current genome. This is, in-part, due to the complexity introduced when assembling a heterogeneous sample containing DNA from multiple psyllids and potentially exacerbated by the use of short reads. To improve quality of genome assembly, we have generated 36.2Gb of Pacbio long reads from 41 SMRT cells with a coverage of 80X for the 400-450Mb psyllid genome. The Canu assembler (Koren 2016) was used to create an interim assembly (Diaci v1.9) with a contig N50 of 115.8kb and 8300 contigs (cSaha et al., 2017). This will be polished with Pacbio and Illumina paired-end reads followed by scaffolding with Illumina mate-pair reads. We are employing Dovetail chicago libraries and 10X Illumina library generated from a single psyllid in conjunction with Bionano optical maps to achieve long-range scaffolding of the genome. This will be the first time all these methods have been applied to resolve an insect genome from a highly heterogeneous sample. The new assembly will be available on https://citrusgreening.org/.

14. **Sequencing of an extremophilic icebug, *Galloisiana yuasai***

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Insects are amongst the most successful and diverse animal groups, spanning over one million described species and having colonized virtually every biotop. Although in recent times the genomic sampling of insect orders has been greatly expanded, there is still a marked lack of data belonging to species that radiated in the early Triassic period, which comprise the majority of wingless insects. This sparseness of available sequencing data is due to inherent difficulties in sequencing organisms possessing both large genome sizes and high levels of heterozygosity, compounded by a lack of available inbred laboratory colonies. We took this challenge and sequenced the genome of the ice-crawler *Galloisiana yuasai* (Notoptera: Grylloblattodea: Grylloblattidae), to the best of our knowledge the first sequenced genome of this clade of wingless cryophilic insects which radiated about 250 mya. The 1.6 Gbp *G. yuasai* draft genome assembly is relatively complete, containing 95.2 % of the Arthropoda-specific BUSCO (Benchmarking Universal Single-Copy Orthologs) gene set and ranks amongst the largest insect genomes sequenced so far. The genome of this wingless insect belongs to a previously unsampled insect order, Grylloblattodea. Its availability will offer new opportunities for molecular-level evolutionary and ecological studies and it will facilitate the investigation of cold-resistance mechanisms in insects.

15. **The sequence of a W-chromosome for the diamondback moth, *Plutella xylostella*, revealed with long-read genome assembly and single chromosome sequencing**

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Females of lepidopteran species are the heterogametic sex and commonly have a WZ chromosome system. Recent studies have begun to unravel the primary molecular mechanism behind this type of sex-determination. We examined this system using a novel genome assembly for the diamondback moth (*Plutella xylostella*) a pervasive pest of cruciferous crops, which is well known for its ability to rapidly acquire resistance to insecticides. In order to combat this challenge, genetically engineered male-selecting strains have been developed by exploiting the sex-alternate

splicing of *doublesex*, which is a downstream target of the sex-determination cascade. Our genome was assembled using PacBio long-read sequencing technology, enabling the resolution of highly repetitive regions, which includes the W-chromosome. In addition, short-read libraries were prepared from laser-dissected W-chromosomes and were utilised, in combination with a published RAD-seq linkage map, to assign contigs to chromosomal locations, resulting in 9.3Mb of W-chromosome sequence. Our findings confirm that the W-chromosome is rich in repetitive sequence and suggest that it also contains a small number of pseudogenes, one of which is a strong candidate for a feminising element. These results suggest a putative evolutionary origin for a dominant feminizing element, however a substantial knowledge gap still remains in lepidopteran species that lack the W-chromosome.

16. Transcriptome analysis and expression of sensory genes in *Aedes aegypti* associated with ultrasonic sound treatment

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The mosquito is a dangerous disease vector, which transfers various diseases like dengue virus, Zika virus, malaria, and West Nile virus. Around the globe, researches on the repellent responses of mosquitoes have intensively studied. This study used an alternative way of mosquito repellent agents rather than chemical repellents such as DEET (N, N-diethly-meta-toluamide). We have employed a novel emitters of ultra-sonic sounds, which showed repellent effects to mosquito behaviors that avoid ultra-sonic sounds. Despite more detailed mechanisms of repellency caused by ultra-sonic sounds needed, ultra-sonic sounds tested here turns out to be one of the powerful repellent resources among the various materials. Using NGS analysis after ultrasonic treatment, we found that some subsets of sensory and other related genes were affected, indicating that ultrasonic sounds affects gene expression associated with host finding behaviors.

17. Genome of the parasitic wasp *Diachasma alloeum*, an emerging model for ecological speciation and transitions to asexual reproduction

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Parasitic wasps in genus *Diachasma* (Hymenoptera: Braconidae: Opiinae) present a promising model for investigating both speciation and the consequences of transitions between sexual and asexual reproductive strategies. We report the recent genome assembly of *Diachasma alloeum* as part of the i5K arthropod genome initiative. Major project goals include: a) characterizing the genome assembly of *D. alloeum,* and b) assessing patterns of molecular rate variation across *Diachasma* genomes.

Native to North America, *Diachasma* wasps are parasites of *Rhagoletis* (Diptera: Tephritidae) flies: wasps lay eggs in fly larvae feeding inside of fruit. Of particular interest, *D. alloeum* is currently experiencing lineage divergence *via* cascading ecological speciation. The speciation of their host *R. pomonella* flies as they shift and adapt to new fruits has induced a parallel incipient speciation event in *D. alloeum*. One axis of adaptation involved in the evolution of reproductive isolation between populations of *D. alloeum* is chemosensation. Wasps use olfactory cues to locate host fruit, which serve as sites for mating and oviposition of eggs into host fly larvae. Wasps searching for flies on different fruits have rapidly evolved differences in fruit odor preferences, warranting the investigation of molecular machinery involved in olfaction and other chemosensory processes as potential contributors to speciation in *Diachasma*. Manually annotated models for odorant, gustatory, and ionotropic receptors in the *D. alloeum* reference assembly will facilitate efforts to survey wasp populations for signatures of recent selection.

Another *Diachasma* wasp species is interesting for a different reason: its loss of sexual reproduction. The asexual wasp *D. muliebre* is geographically isolated from its sexual relatives, and evidence suggests this species originated as a single loss-of-sex event perhaps as recently as 10,000YA. Loss of sex in *D. muliebre* may have profound effects on the generation and maintenance of genetic variation and subsequent adaptive potential. We evaluated whether the asexual *D. muliebre* has retained the mechanistic capacity for meiosis, a process essential for sexual reproduction. We used a *de novo* genome assembly for *D. muliebre*, which was then queried using a multi-gene "meiotic toolkit" inventory whose products function in meiosis. Manual annotation of coding regions indicated no loss of any meiosis-critical genes in *D. muliebre* compared with *D. alloeum*. Moreover, we found no evidence of gene degradation *via* relaxed selection in *D. muliebre*, indicating that these asexual wasps may retain the capacity to engage in meiosis. Distance-based phylogenetic methods comparing meiosis-specific coding regions in *Diachasma* supported topologies where sexuals and asexuals have no significant differences in evolutionary rates. Future work includes the examination of larger gene datasets, including neutrally-evolving regions such as introns, to assess whether broad differences in genome-wide evolution rates exist as a direct consequence of asexuality.

18. Metabolomics of diapause in *Aedes albopictus*

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Diapause is an alternative life history strategy that allows insects to align their growth with seasonally favorable conditions. This adaptation plays a critical role in shaping the geographic distribution and seasonal abundance of numerous vector species, and therefore affects the risk of arboviral infection. While the adaptive and epidemiological significance of diapause is clear, little is known about the molecular mechanisms regulating this trait. *Aedes albopictus* is an emerging model system for investigating the molecular regulation of photoperiodic diapause. *Ae. albopictus* is a highly invasive and aggressive human-biting vector for several arboviruses including Dengue, Zika, and Chikungunya. Photoperiodic diapause has facilitated the invasion of *Ae. albopictus* across temperate regions in the United States and Europe, putting millions of people at risk for endemic arboviral transmission. I performed untargeted metabolomics to compare the metabolite signature of age-matched diapause and non-diapause *Ae. albopictus* eggs. My analysis of these data integrates previously obtained RNA-seq results on genome-wide expression patterns to provide multi-faceted insight into mechanisms of diapause regulation. My integrated approach identified coordinated shifts in both transcriptional and metabolic
pathways known to regulate juvenile hormone III (JH3) abundance, a key mediator of diapause
maintenance in mosquitoes. Additionally, I identified metabolomics shifts related to lipid accumulation, shifts in metabolic activity, and increased stress tolerance.

19. Regulation of population size in facultative endosymbionts of the pea aphid

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In vertically transmitted endosymbiosis, the host interfaces with a coevolving symbiont population. Population size is a key factor in symbiont function and therefore host ecology. Large symbiont population sizes promote selection for traits that benefit the symbiont over the host, while small symbiont populations increase host level efficiency of selection at the risk of drift in the symbiont. In the pea aphid *Acyrthosiphon pisum*, host strains with smaller primary symbiont populations have higher fitness than strains with larger symbiont populations.
To examine the relative contributions of host and symbiont processes on symbiont population
size, we use reciprocal transplants of the facultative symbionts *Serratia symbiotica* and *Hamiltonella defensa* in pea aphid strains. Symbiont population sizes will be approximated using absolute qPCR to measure the abundance of bacterial and host genomes. Population sizes are expected to differ primarily by bacterial species rather than host lineage. This would indicate bacterial control over population size, or a conserved host response to each bacterial species reflecting less fine-tuned host control of facultative symbionts than obligate symbionts.
Characterizing the relative contributions of host and symbiont in regulating population sizes
contributes to understanding the mechanisms setting the parameters of host-symbiont coevolution.

20. Untangling the transmission dynamics of primary and secondary vectors of *Trypanosoma cruzi* in Colombia: parasite infection, feeding sources and discrete typing units

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Trypanosoma cruzi is the causative agent of Chagas disease. Due to its genetic diversity has been classified into six Discrete Typing Units (DTUs) in association with transmission cycles. In Colombia, natural *T. cruzi* infection has been detected in 15 triatomine species. There is scarce information regarding the infection rates, DTUs and feeding preferences of secondary vectors. Therefore, the aim of this study was to determine *T. cruzi* infection rates, parasite DTU, ecotopes, insect stages, geographical location and bug feeding preferences across six different triatomine species.

A total of 245 insects were collected in seven departments of Colombia. We conducted molecular detection and genotyping of *T. cruzi* with subsequent identification of food sources. The frequency of infection, DTUs, TcI genotypes and feeding sources were plotted across the six species studied. A logistic regression model risk was estimated with insects positive for *T. cruzi* according to demographic and eco-epidemiological characteristics.

We collected 85 specimens of *Panstrongylus geniculatus*, 77 *Rhodnius prolixus*, 37 *R. pallescens*, 34 *Triatoma maculata*, 8 *R. pictipes* and 4 *T. dimidiata*. The overall *T. cruzi* infection rate was 61.2% and presented statistical associations with the departments Meta (OR: 2.65; 95% CI: 1.69-4.17) and Guajira (OR: 2.13; 95% CI: 1.16-3.94); peridomestic ecotope (OR: 2.52: 95% CI: 1.62-3.93); the vector species P. geniculatus (OR: 2.40; 95% CI: 1.51-3.82) and T. maculata (OR: 2.09; 95% CI: 1.02-4.29); females (OR: 2.05; 95% CI: 1.39-3.04) and feeding on opossum (OR: 3.15; 95% CI: 1.85-11.69) and human blood (OR: 1.55; 95% CI: 1.07-2.24). Regarding the DTUs, we observed TcI (67.3%), TcII (6.7%), TcIII (8.7%), TcIV (4.0%) and TcV (6.0%). Across the samples typed as TcI, we detected TcIDom (19%) and sylvatic TcI (75%). The frequencies of feeding sources were 59.4% (human blood); 11.2% (hen); 9.6% (bat); 5.6% (opossum); 5.1% (mouse); 4.1% (dog); 3.0% (rodent); 1.0% (armadillo); and 1.0% (cow).

New scenarios of *T. cruzi* transmission caused by secondary and sylvatic vectors are considered. The findings of sylvatic DTUs from bugs collected in domestic and peridomestic ecotopes confirms the emerging transmission scenarios in Colombia.

21. Anopheles genome assembly improvements guided by evolution

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Results from the *Anopheles* 16 genomes project and other initiatives have made publically available the genome assemblies of more than 20 anophelines. In contrast to the highly contiguous and near-completely chromosomally mapped *Anopheles gambiae* PEST genome assembly, the initial draft assemblies of the more recently sequenced anophelines vary greatly in their contiguity and only a handful have at least partial chromosomal assignments. Ongoing assembly improvement strategies including generating additional sequencing and/or physical mapping data continue to improve these initial drafts, e.g. 98% of the highly contiguous *Anopheles albimanus* assembly is now anchored to chromosomes. Here we employ four different computational approaches that each attempt to characterise evolutionary signatures from analysing the conservation of gene order - synteny - to identify putative scaffold adjacencies in the current assemblies. Leveraging information from cross-species comparisons enables the delineation of well-maintained synteny blocks, which provide support for scaffold adjacencies that reconstruct the conserved arrangements in assemblies where orthologues are located at scaffold extremities. These predictions can be validated through comparisons with independent evidence, where available, such as physical mapping data, or mapped RNAseq-derived transcripts that link scaffold extremities, or indeed *de novo* assemblies from new sequencing data. The consensus predictions offer evolutionarily well-supported sets of scaffold adjacencies that lead to the improved contiguity of draft assemblies without the associated costs or time investments required for additional sequence-based support. Thus, whether employed as supporting data for experimentally based assembly improvement approaches, or as complementary data to further enhance such improvements, or as stand-alone evidence as part of an assembly-building pipeline, these evolutionarily guided approaches offer a handy new set of utensils in the genome assembly toolbox.

22. Dynamic of *Spodoptera frugiperda* chromatin histones marks regarding different life stages

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Spodoptera frugiperda (Lepidoptera, Noctuidae) is a major agricultural pest in the Americas. At larval stage it defoliates crops such as corn, sorghum or soybean.,Two S. frugiperda strains have been described in the scientific literature based on their diet preference: a corn (sf-C) and rice (sf-R) strain. Although differentiation seems to appears at a genetic but also physiologic level [1,2,3], the strain vs. species denomination is still in discussion because both of them look physically alike and can mate to produce viable hybrids [4]. Our work aims to understand the functional genomic differences between sf-C and sf-R and to determine if the adaptation to host-plant is driving the differentiation between the two strains. My PhD project focuses particularly on epigenetic differences. During my first year phD I studied the role of negative gene regulators known as microRNA in the two strains. Now, I'm searching for the presence of repressing and activating chromatin histone marks (respectively H3K27me3, H3K9me3 and H3K4me3) and its plasticity regarding different life stages. Eggs, larvaes, pupaes and also moths are screened for this purpose.

My current goal is to adapt the chromatin immunoprecipitation (ChIP) sequencing protocol to *Spodoptera frugiperda*. Differents methods are tested to recover the maximum DNA sequences in terms of quantity/quality associated to the epigenetic mark. For further work, we'd like to compare the chIP-seq datas between strains to assess their respective particularity but also combine these results with RNA-seq, FAIRE-seq but also microRNA-seq analysis data [in writing] conducted by our team to get a global view on functional genomic regulation at n°4 larval stage (L4). Finally a major interest will be to compare these chIP-seq analyses with the ones performed in other arthropods like the *Drosophila* model organism or more closely related lepidopteran like *Heliconus Erato* [5].

23. Aphid Transcriptomic Analysis and the Development of Integrated Pest Management Strategies in Greenhouse Crops

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Aphids, in addition to being one of the most economically important insect pests in North America, are rapidly emerging as a robust model for studying phenotypic plasticity and responses to evolutionary pressures at the genomic level. Aphid natural enemies, such as ladybird beetles (Coleoptera: Coccinnelidae) or parasitoid wasps (Hymenoptera: Braconidae), are commonly used as an alternative to pesticides in greenhouses. These biocontrol methods are generally very effective, but we believe that when applied liberally and combined with the closed environment of a greenhouse such robust selection pressures create an ideal environment for the development of resistant aphid genetic lines. Using established methods for transcriptome analysis, comparative genomics, and chemical ecology, we hope to be able to track the specific epigenetic and physiological changes which occur across asexual generations of two common greenhouse pest species of aphids, the green peach aphid (*Myzus persicae*) and the mealy cabbage aphid (*Brevicoryne brassica*), when exposed to various greenhouse biocontrol strategies. With this data we hope to be able to better inform growers about the efficacy of these biocontrol strategies, and their viability with respect to long-term use.

24. Proteomic analysis of the midgut and malpighian tubules of laboratory and field strains of *Aedes aegypti* from Colombia.

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Dengue, chikungunya and Zika viruses are considered a major public health threat in Colombia. These arboviruses are primarily transmitted by *Aedes aegypti*. In these mosquitoes Midgut (MG) and Malpighian tubules (MT) are important tissues because they play a crucial role in processing the sugar and blood meal. In addition, the MG is responsible not only for digestion, it also the first site of contact of potential pathogens as viruses, parasites and bacteria, during the infection process. On the other hand, in the Midgut and the Malpighian tubules, a general enrichment in the transcription of genes from major detoxification gene families related to insecticide resistance was observed. In this study, a comparative proteomic analysis was performed using TMT6xplex- Isobaric tags labelling and Liquid chromatography–Tandem mass spectrometry (LC-MS/MS), in order to identify proteins differentially expressed in the Midgut and Malpighian tubules of *Ae. aegypti* in three laboratory and three field strains of *Aedes aegypti*, with different phenotypes (Resistant or susceptible) to pyrethroids insecticides.

Using the software proteome discoverer and Mascot MS/MS ion search, 438 proteins were detected in these tissues. According to gene ontology analysis, the majority of proteins found in the MG and MT participate in catalytic activities (34%) and amino acid metabolism (63%). Others proteins related to dengue and chikungunya viruses infection were found as well (Enolase, Histona 2A, Histona 2B and Actina).

In terms of the expression of proteins, comparisons between strains, showed that laboratory strains are more homogeneous than the field strains. This could be possible consequences of laboratory colonization on the phenotypic and genotypic variation of a mosquito population. One protein was upregulated in the laboratory resistant strains while 60 proteins were upregulated in the resistant field strains. The Only protein upregulated in the laboratory resistance strains was identified as Ribosomal protein L25/L23 (Accession number AAEL017516-PA).

Sixty proteins upregulated were found in the resistant field strains when compared to susceptible field strain. The most of these proteins (67%) were related to translation and ribosome structure. Some of these ribosomal proteins have been found in deltamethrin resistant *Culex pipiens* (other culicidae with medical importance).

We found that the Microsomal glutathione s-transferase, which is part of *Ae. aegypti*
Glutathione transferase (GST) family, was upregulated in the resistant field strains. Insects GSTs have been implicated in resistance to insecticides through direct metabolism of insecticide, sequestration, or by protecting against secondary toxic effects, such as increases in lipid peroxidation, induced by insecticide exposure.

When comparing the expression of the proteins among the resistant field strains, we found that one protein identified with Vector Base accession number AAEL003634 was not expressed in one of then resistant strain. Using blastp (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the VectorBase Data Base (https://www.vectorbase.org/search/site/AAEL003634) the protein was identified as "Hsp70 Heat shock". These proteins are an important modulator of stress in insects and have been associated with to dengue virus susceptibility and chikungunya viruses
infection.

This is the first proteome study of the Midgut and Malpighian tubules in *Ae. aegypti* in Colombia and it provides insights about the proteins found in these tissues that could be related to insecticide resistance and arboviruses infection.

25. The male fertility gene *kl-3* is linked to the Y chromosome of the kissing bug *Triatoma infestans*.

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In many insects, the Y chromosome plays a key role in sexual determination and male fertility. The Chagas disease vector *Triatoma infestans* has 22 autosomal chromosomes and a pair of XY sex chromosomes. However, the knowledge on the Y chromosome of this species, its genetic content or its biological function, is very poor. Due to repetitive DNA, Y chromosome sequences are poorly assembled in genome projects, hindering structural and functional studies on Y-linked genes. Our group has made important contributions in this field and we have recently identified 9 genes linked to the Y chromosome of *Rhodnius prolixus*. In the present study, we use the YGS script to identify, for the first time, Y linked genes in *T. infestans*. One of the genes found encodes a γ-dynein heavy chain, homologous to the *Drosophila melanogaster* Y-linked gene *kl-3*. In *D. melanogaster*, the dyneins of the Y chromosome are known as male fertility factors and their deletion causes male infertility. Our results also suggest that there are two γ-dynein sequences in the *T. infestans* genome. One of the sequences appears to be complete in an autosomal scaffold, whereas the Y-dynein is dispersed in several scaffolds (a typical pattern of Y-genes in assembled genomes). An evolutionary analysis of the γ-dynein genes suggests that the Y γ-dynein of *T. infestans* is, indeed, orthologous to the *kl-3* gene of *D. melanogaster*. This is the first report of a *kl-3* orthologue linked to the Y chromosome of an insect species outside the diptera clade. In addition to the first report of a Y-linked gene in *T. infestans*, this finding is of great relevance for the study of the evolution of Y chromosomes, since these data suggest that *kl-3* has moved independently to the Y chromosomes of *Drosophila* and *T. infestans*, an unusual event that corroborates with a theory that the gain of genes has an important role in the evolution of Y chromosomes.

26. Genome sequencing, assembly and annotation of the spider *Dysdera silvatica* Schmidt, 1981 (Araneae, Dysderidae). A resource for the study of an adaptive radiation in the Canary Islands.

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Oceanic island biotas have been long recognized as simplified natural experiments of evolution, providing tractable case studies for assessing the genomic determinants of biodiversity. The woodlouse-hunter spider genus *Dysdera*

(Araneae) is one of the most spectacular examples of species diversification on islands[1]. Canary Islands harbors 46 endemic species, for about 200 species in the mainland[1]. Using the terrestrial radiation of *Dysdera* in the Canary Islands as a model system, we aim to find the genomic signatures associated with species diversification as well as those responsible of the specific dietary shift[3] (ecological specialization) undergone by this genus.

After determining by flow cytometry the genome size of *D. silvatica* (approx. 1.7 Gbp), we sequenced this genome by combining different strategies and sequencing platforms. We used illumina short-reads, both paired-end (PE) and mate-pair (MP; 5Kb) libraries, resulting in approximately 30x coverage each, and single molecule real time sequencing (PacBio approximately 5x estimated coverage) in a de novo assembly approach. We first estimated the best *k*-mer size for our data using KmerGenie[4] and performed the initial assembly using the PE library in SOAPdenovo2[5]. MP reads were then used to run a scaffolding step in the same software and several rounds of gap filling were applied to the resulting assembly using PBJelly[6] and PacBio reads. In order to improve the assembly continuity of gene regions and to carry out genome annotation, we are using the available RNA-seq data for this species (also generated by our group[7]) to perform a transcript-guided scaffolding and to identify and characterize the structure of all expressed genes.

Finally, in parallel with the assembly and annotation of the *D. silvatica genome*, we have proceeded to the low coverage sequencing (approx. 10x) of four additional genomes, all of them from closely related *Dysdera* species, which will allow us to identify and characterize the genomic determinants of the adaptive radiation of this genus in Canary Islands.

27. From single genomes to population genomics, iterative improvement of genome annotation for multiple vector species at VectorBase.

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VectorBase is an NIAID-funded Bioinformatic Resource Center focused on invertebrate vectors of human pathogens which hosts data from 38 vector species including those responsible for transmission of malaria and many important viral pathogens such as dengue, yellow fever. The initial focus of the project was the first-pass annotation of newly sequenced genomes in collaboration with large sequencing centers. The development of new sequencing technologies and concomitant reduction in cost has led to many genomes being sequenced and a focus on rapid data generation rather than reference quality,. Genome annotation has not kept pace with these changes but the requirements for high quality annotations remains for many functional studies. We will discuss the transition to rapid first-pass annotation strategies supplemented by iterative community-based ones using tools like Apollo to improve and supplement the reference gene sets for vector genomes. Further we consider the challenges involved with improvement of the genome assemblies as second, or third iterations for key species become available through novel sequencing and assembly protocols.

28. VectorBase: A bioinformatics resource for invertebrate vectors and other organisms related with human diseases

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VectorBase.org is a free, web-based bioinformatics resource center (BRC), for invertebrate vectors of human pathogens. 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*). For most of these 40 genomes, VectorBase provided the genome wide automated annotation and currently maintains the software Web Apollo for scientist working on gene manual annotations. This database also has transcriptomes (microarrays, RNAseq), proteomes (mass spectrometry) and population data (SNPs, microsatellites, insecticide resistance and other genotypes and phenotypes) for an even wider list of species. Primary data is imported from external databases, directly submitted by users or generated and computed by VectorBase. For example, we calculate homology relationships i.e., paralogs and orthologs, the effect of sequence variations on protein coding sequences, and the mapping of experimental transcript and peptide evidence to gene models. Orthology relationships are used to project gene descriptions from species that have been extensively studied to other closely-related but less well described species. The hosted data has been used for basic and translational research using data in new or re-purpose analyses, descriptions and hypotheses testing. Raw and processed data can be exported or download in a variety of different formats, visualized, browsed, queried and analyzed with on-site or external tools. In the last year, two new tools provided the ability to look for samples with SNP and INDEL genotyping calls or search and explore metadata associated with biological samples and display them in the Genome Browser and the Population Biology Browser. 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(s) 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. This BRC is NIAID/NIH-funded.

29. Improvements to the *Lucilia cuprina* draft genome and transcriptome

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Lucilia cuprina is a parasitic blowfly of major global importance to the livestock industry. Maggots of this fly parasitize the skin of animal hosts, feed on excretions and tissues, and cause severe disease (flystrike or myiasis). In Australasia alone, flystrike in sheep causes productivity losses estimated at $320 million per annum. Although there has been considerable research on flystrike, little is understood about the molecular biology, biochemistry and genetics of this parasitic fly as well as its relationship with the host animal. No vaccine is available and resistance in blowfly against almost all available treatments demands new and innovative interventions. The sequencing, annotation and analyses of the draft genome of *L. cuprina* was an exciting possible answer to blowfly control and provided a critical foundation for which to build upon. Recent improvements to the genome, incorporating Dovetail Genomics Chicago sequencing + HiRise data, PacBio sequencing, and transcriptomic data from multiple life cycle stages and conditions, has resulted in a drastically refined 'draft 2' genome. In the near future, we will be able to test the function of genes and gene products of major biological significance using CRISPR, which should facilitate the development of novel interventions against flystrike.

30. Differential transcriptomic profiles in honey bee *Apis mellifera* workers under brood rearing-suppressed condition

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Perturbation of normal behaviors in honey bee colonies by any external factor can immediately reduce the colony's capacity for brood rearing, which can eventually lead to colony collapse. To investigate the effects of brood-rearing

suppression on the biology of honey bee workers (nurses and foragers), gene-set enrichment analysis (GSEA) of the transcriptomes of worker bees with or without suppressed brood rearing was performed. When Free Nurse (actively engaged in nursing) and Free Forager (actively engaged in foraging) individuals were compared, both the labor-specific pathways predicted from GSEA and the expression profiles of some genes in the neuroactive ligand-receptor interaction pathway reflected the physiologies that can be naturally expected from worker bees with respective labor roles. When brood rearing was suppressed, pathways associated with both protein degradation and synthesis were simultaneously over-represented in both Net Nurse (nursing activity suppressed) and Net Forager (foraging activity suppressed) individuals, and their overall pathway representation profiles resembled those of normal foragers and nurses, respectively. These findings suggested that active reformation of worker bee physiology and labor reversion occur when the normal labor of worker bees is obstructed. Over-representation of the notch signaling pathway in Net Forager relative to Free Forager implied induction of neural plasticity upon the disturbance of foraging activity. Some genes in the neuroactive ligand-receptor interaction pathway associated with the regulation of neuronal excitability, cellular and nutritional stress and aggressiveness were over-expressed in both foragers and nurses under brood rearing suppression perhaps to manage in-hive stress under unfavorable conditions.

31. Using engineered yeast expressing interfering RNA larvicides to control *Aedes aegypti*

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The *Aedes aegypti* mosquito transmits multiple arboviruses responsible for significant human morbidity and mortality globally, including dengue, yellow fever, Zika, and chikungunya. As effective vaccines are generally lacking and no drug treatments exist, disruption of arbovirus transmission primarily relies on mosquito control using broad spectrum pesticides. Regrettably, an increasing number of studies show selection for resistance of *Ae. aegypti* to these chemicals, and their potential impacts on non-target species are of ongoing concern. The use of RNA interference (RNAi), which allows for targeted silencing of gene expression in *Ae. aegypti,* could provide highly effective new biorational larvicides while limiting the impacts on non-target organisms. Knowledge of known larval lethal genes in other insects combined with the recent sequencing of multiple disease vector mosquito genomes has facilitated our ongoing RNAi screens for novel mosquito larvicides. In an effort to identify cost-effective RNA production systems and mechanisms for delivery of interfering RNA to mosquito larvae, we engineered baker's yeast, *Saccharomyces cerevisiae*, to produce and propagate short hairpin RNAs corresponding to two larval lethal genes identified in a recent *Ae. aegypti* RNAi screen. Feeding *Ae. aegypti* larvae with the engineered yeast induces >90% larval mortality by silencing target gene expression and disrupting neural development. We then developed and characterized larvicide yeast granules for future semi-field and field experiments. Yeast granules retained their larvicidal activity when heat inactivated, worked well in various water volumes, and also attracted significantly more gravid females to lay their eggs than water alone. The low cost of production, species specificity, and efficacy make engineered yeast a suitable tool to target *Ae. aegypti* as part of an integrative pest management program.

32. Light manipulation of mosquito behavior: Acute and sustained photic suppression of biting in the *Anopheles gambiae* malaria mosquito

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Host-seeking behaviors in anopheline mosquitoes are time-of-day specific, with a greater propensity of biting occurring during the dark phase of the light:dark (LD) cycle. We investigated how a short exposure to light presented during the night or late day can inhibit biting activity and modulate flight activity behavior. *Anopheles gambiae* s.s.

maintained on a 12:12 LD cycle, were exposed to white light at the onset of night and the proportion taking a blood meal in a human biting assay was recorded every 2 hours for 8 hours. The pulse significantly reduced biting propensity in mosquitoes for up to 4 hours following administration, and with no differences detected after 6 hours. Conversely, biting levels were significantly elevated when mosquitoes were exposed to a dark treatment during the late day, suggesting that light suppresses biting behavior even during the late day. These data reveal a potent effect of a discrete light pulse on biting behavior that is both immediate and sustained. We expanded this approach to develop a method to reduce biting propensity throughout the night by exposing mosquitoes to a series of 10-minute pulses presented every 2 hours. We reveal both an immediate suppressive effect of light during the exposure period and 2 hours after the pulse. This response was found to be effective during most times of the night: However, differential responses that were time-of-day specific suggest an underlying circadian property of the mosquito physiology that results in an altered treatment efficacy. We then examined the immediate and sustained effects of light on mosquito flight activity behavior following exposure to a 30-minute pulse, and observed activity suppression during early night, and elevated activity during late night. To begin to understand the molecular basis for these sustained effects of light on behavior we have initiated a genomic analysis of photic-induced expression changes in the mosquito. As mosquitoes and malaria parasites are becoming increasingly resistant to insecticidal and drug treatments, there is a necessity for the development of innovative control strategies beyond ITNs. These data revealing the potent inhibitory effects of light exposure and the utility of multiple photic pulses presented at intervals during the night/late day, may prove to be an effective tool that complements established control methods.

33. Genome-Wide Profiling of Diurnal Rhythmic Gene Expression in the Water Flea *Daphnia Pulex*

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Marine and freshwater zooplankton exhibit daily rhythmic patterns of physiology and behavior which may be regulated by the LD cycle and/or endogenous circadian clock. One of the best-studied zooplankton taxa, the freshwater crustacean *Daphnia*, has a 24-hr diel vertical migration (DVM) whereby the organism travels up and down through the water column daily. DVM plays a critical role in resource tracking and the avoidance of predators and ultraviolet radiation. However, there is little information at the transcriptional level linking the expression patterns of genes to the rhythmic physiology/behavior of Daphnia. We therefore sought to perform a genome-wide temporal transcriptional analysis on *D. pulex*. Whole bodies were collected every 4 hr over 44 hr under LD cycle diel/diurnal conditions, RNA hybridized to microarrays and data analyzed using the JTK_CYCLE algorithm. From the 21,002 genes with expression levels above background, we identified 1,661 rhythmically expressed genes using a conservative q-value ($q < 0.1$; $p < 0.03$) and 22-26-hr period length cutoff criteria. These genes represent 5.7% of the total *D. pulex* gene-set, and 7.9% of genes considered expressed. Using a comprehensive network modeling and analysis approach, we identified an additional 133 co-regulated rhythmic genes that have similar network topological properties as those identified by JTK_CYCLE. This network approach also provided novel functional annotations for 374 genes. The rhythmically expressed genes possess diverse biological functions including immunity, oxidative detoxification, and sensory processes. We identified differences in the chronobiology of *D. pulex* as compared to other well-characterized terrestrial arthropods, including an apparent lack of rhythmicity in several of the expected-rhythmic gene families, e.g. in metabolic pathways and visual transduction. Furthermore, while *D. pulex* possess a full complement of the canonical clock genes found in other animals, the expected synchronized, rhythmic expression of these genes was not apparent. Our results reveal that Daphnia exhibits an abundance of rhythmic gene expression, and adds to a growing body of literature suggesting that the genetic mechanisms governing rhythmicity in crustaceans may be divergent from other arthropod lineages, including insects.

34. The methylome of the marbled crayfish, a novel model system for epigenetics

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Methylation of cytosine residues in CpG dinucleotides carries out diverse roles including epigenetic regulation of gene expression and genome stability. Methylation patterns differ between vertebrates and invertebrates, and their roles in evolutionary and environmental adaptation remain unclear. The subphylum Crustacean shows a high morphological and ecological diversity, but few crustacean methylomes have been published so far. The marbled crayfish *Procambarus virginalis* is a triploid organism which is the only known obligatory parthenogen among the decapod crustaceans. Despite its genetic uniformity, it shows a high degree of phenotypic variation and occurs in a variety of ecological environments. A draft genome assembly was recently completed by our group and encodes the complete toolkit for DNA methylation, including homologues for Dnmt1, Dnmt3, and Tet. Mass-spec and whole-genome bisulfite sequencing (WGBS) showed global methylation levels of 2.5%. WGBS revealed that methylation is CpG-specific, bimodally distributed and symmetric on both strands, which is characteristic for animal methylomes. Further analyses showed that methylation was mainly targeted to the gene bodies of housekeeping genes and subclasses of repeats. The absence of confounding genetic variations and its suitability as a laboratory animal establishes the marbled crayfish as a unique model system for the study of invertebrate epigenetics. Future studies will include the analysis of epigenetic variants in the context of environmental adaptation.

35. The *Gammarus* genome project: building a consortium for challenging the genome diversity of a crustacean family of ecotoxicological importance.

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The species *Gammarus fossarum* and *Gammarus pulex* constitute abundant and widespread European freshwater scuds. They belong to gammarids, a heterogeneous family of crustacean amphipods which are largely distributed in the northern hemisphere. A number of cryptic species have been described in this species complex, but the complexity of their phylogeny needs further studies. A great variety of vulnerability to environmental contamination, notably heavy metals, has been observed in different populations in French rivers. Importantly, gammarids are a well-established ecotoxicological model used in aquatic toxicology, particularly in Europe. Our recent works show the strength of the proteogenomics approach to increase the molecular knowledge of the reproductive and endocrine signaling pathways in *Gammarus fossarum*. These results are contributing to the development of new promising molecular biomarkers of endocrine disruption in freshwater crustaceans in order to assess the quality of water and the endocrine disruptive potential of emerging contaminants.

Here we present the work in progress to establish a consortium that will address the genome sequencing of *Gammarus fossarum* taking into account the natural population diversity, as well as some preliminary results so far obtained. We anticipate that the *Gammarus* genome project will provide a valuable resource that will facilitate the development of ecotoxicogenomics approaches in environmental risk assessment.

36. Biosurveillance of Alien Forest Enemies (bioSAFE) – creating new genomic tools to meet the challenges posed by forest alien invasives

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The world's forests face unprecedented threats from invasive insects and pathogens. This threatens the ecological and economic stability of our natural and urban forests. New introductions and interceptions of Forest Invasive Alien Species (FIAS) are escalating at an alarming rate and managing this risk requires vigilant biosurveillance. Prevention and early detection are keys to successful biosurveillance programs, but are challenging to achieve. We will address these challenges by developing a biosurveillance pipeline to rapidly generate genomics tools that will provide: 1) accurate identification; 2) assignments to source populations and invasion pathways; 3) identification of Fitness and Outbreak-related Epidemiological (FORE) traits that can impact invasion outcomes; 4) reduced uncertainty of invasion outcomes and can inform decision support systems; 5) transferable biosurveillance tools to end users. In Canada four FIAS have been identified as current and urgent threats: Asian longhorned beetle, Dutch Elm disease fungi, Sudden oak death pathogen, and Asian gypsy moth. We will develop a biosurveillance pipeline using genomic tools developed for these four species to speed up and inform novel decision-support systems for FIAS mitigation and management. Keywords: diagnostics, epidemiology, WGS, GBS, target enrichment

37. Two sand fly genomes:*Phlebotomus papatasi* and *Lutzomyia longipalpis*

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Sand flies serve as vectors for several established, emerging and re-emerging infectious agents. As important vectors of human disease, phlebotomine sand flies are of global significance to human health, transmitting protozoan, bacterial, and viral pathogens, the most devastating which is leishmaniasis. Here we sequenced the genomes of two different phlebotomine sand flies, *Phlebotomus papatasi* and *Lutzomyia longipalpis*, that exhibit distinct distributions, behavior, and pathogen specificity. *P. papatasi* is a restrictive vector, transmitting only *Leishmania major* parasites, is widely distributed in the Old World and resides in variable habitats. *Lu. longipalpis* is found only in the New World, distributed throughout Central and South America. Although considered a permissive vector in laboratory conditions, this species only transmits *Le. infantum* naturally. The habitat of this vector differs from *P. papatasi*, preferring primarily forested habitats. In addition to characterizing the two genomes we performed whole-genome sequencing and alignments of individual flies collected from 3 locations across the *P. papatasi* distribution. *Lu. longipalpis* is thought to be a complex of cryptic sibling species that have a complicated sexual ecology involving an intricate interaction of courtship behavior (including acoustic signals), mate preference, aggression, host attraction, and pheromones. Here we performed whole-genome sequencing and population genetic analysis of 5 populations of *Lu. longipalpis* that exhibit different mating songs and pheromone specificities.

38. Chromosome level assembly of the *Apis dors*ata and *Apis florea* genome

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The *Apis* genus (honey bees), a group of "advanced eusocial" corbiculates bees characterized by honey production and wax comb-based nesting, is a vital model for the study of evolution of eusociality. Since the publication of the genome

of the honey bee *Apis mellifera* (subgenus *Apis*) in 2006, the genomes of three other honey bees have been sequenced. They are valuable genomic resources providing insights into key features of eusocial Hymenoptera. However, in contrast to the assembled chromosome sequences of the *Apis mellifera*, the genomes of the representatives of two important subgenera, the giant bee *Apis dorsata* (subgenus *Megapis*) and the draft bee *Apis florea* (subgenus *Micrapis*), have been assembled only at scaffold level. To address this problem, here we report the process of constructing chromosome-level genome assemblies of *Apis dorsata* and *Apis florea* on basis of the recently published genetic linkage maps of those two species. Using 1189 SNP markers for *Apis dorsata* and 1279 SNP markers for *Apis florea*, we oriented and arranged unplaced scaffolds of previous assemblies into chromosomes. These new assemblies will provide additional materials for comparative genomics between honeybee species and facilitating research on eusociality.

39. Community annotation across 26 non-model arthropod species

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Manual annotation can improve the value and accuracy of computationally predicted gene models. Non-model genomes with small research communities usually lack resources to hire dedicated curators for manual annotation. Community annotation can harness the expertise of the scientific community for genomes with fewer curation resources. Under the purview of the i5k pilot project, we asked whether 1) community manual annotation of non-model genomes be performed across many genomes, and 2) what level of manual annotation can a distributed community achieve? We find that, given the appropriate setup, large communities can manually annotate across many non-model organisms, and that the nature of the manual changes can be assessed computationally. However, identifying the biological validity of the changes to the manually annotated models at scale is challenging.

40. HymenopteraMine at Hymenoptera Genome Database: An Efficient and Customizable Data Mining Resource for Improved Genomic Analysis

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HymenopteraMine is the data mining resource for the Hymenoptera Genome Database (HGD; http://hymenopteragenome.org), which hosts genomes of bee, wasp and ant species. The goal of HymenopteraMine is to accelerate genomics analysis by enabling researchers without scripting skills to create and export customized annotation datasets merged with their own research data for use in downstream analyses. HymenopteraMine uses the InterMine platform to integrate data from a variety of sources, including genome assemblies, genes (RefSeq, Official Gene Set), proteins (UniProt), protein families and domains (InterPro), homologs (OrthoDB), pathways (KEGG), Gene Ontology (UniProt-GOA), variation (dbSNP) and publications (PubMed). By including fly homologs, HymenopteraMine allows users to leverage the Reactome pathways and BioGRID interactions that have been curated for *Drosophila melanogaster*. HymenopteraMine also includes pre-computed variant effects and RNAseq-based transcript expression levels for *A. mellifera*.

HymenopteraMine provides simple and sophisticated data mining tools. Built-in template queries serve as starting points for data exploration, while the QueryBuilder tool supports construction of custom queries. The List Analysis and Genomic Regions search tools execute queries based on uploaded lists of identifiers and genome coordinates, respectively. HymenopteraMine facilitates cross-species data mining based on orthology and supports meta-analyses by tracking identifiers across gene sets and genome assemblies. User tutorials are available in written and video format.

41. Targeted next-generation sequencing of detoxification genes in *Culex pipiens* complex mosquitoes: Discovery of SNPs and copy number variants associated with insecticide resistance

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Insecticide resistance in vector mosquitoes can increase the frequency with which insecticides are applied, require more insecticide to be used and reduce the ability of vector control districts to reduce mosquito populations during a disease outbreak. Using Life Technology's Ampliseq process, we developed a panel of 115 genes known or hypothesized to be involved in insecticide resistance. The panel is run on an Ion Torrent PGM sequencer. Individual families of *Culex quinquefasciatus* were tested for resistance to Permethrin and Malathion using a bottle bioassay. Data were analyzed by comparing resistant to susceptible individuals (n= 106 unrelated individuals). We conducted analyses to discover two kinds of genetic changes associated with resistance: SNPs (single nucleotide polymorphisms) and CNVs (copy number variants). Genes with statistically significant SNPs included the gene associated with kdr (knock down resistance), as well as esterases, glutathione S-transferases and cytochrome P450s. Similarly, genes shown to exist in increased copy numbers included two esterase genes known to be duplicated in individuals showing metabolic resistance, as well as the gene associated with kdr, and several cytochrome P450 genes. These results will be used to develop PCR based assays to better characterize resistance in field-collected mosquitoes. We are also using the panel to sequence *Culex pipiens* from the U.S., and *Culex pipiens* form molestus from the U.S. and several locations internationally to 1) develop an updated assay to distinguish *Cx. pipiens* from *Cx. quinquefasciatus* in the U.S. and 2) conduct a phylogenetic analysis of U.S. and international specimens of *Cx. pipiens* form molestus.

42. Analysis of insect *Kirre/Roughest* and *Sticks and stones/Hibris* paralogous groups reveals concurrent gene duplication events for both ligand and receptor genes in Dipteran lineages.

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Muscle development requires a cascade of genetic interactions that leads to the specification of both general and individual muscle characteristics. In *Drosophila melanogaster,* the genes *Kirre*, *Sticks and Stones (Sns)*, *Hibris (Hbs)*, and *Roughest (Rst)* regulate the fusion of founder cells (FCs) with fusion competent myoblasts (FCMs) to generate a mature muscle. Specifically, *Kirre* and *Rst* are paralogous genes that code for cell-surface proteins expressed in FCs, while *Sns* and *Hbs* are paralogous genes expressed in FCMs that code for cell-surface ligands that interact with Kirre and Roughest proteins. During adult muscle development, the expression of *Kirre* requires signals from the innervating motor neurons that then regulates myoblast accumulation and fusion. However, the roles of these genes in other insects is unknown. In the moth *Manduca sexta,* some adult muscles can develop without innervation and thus may not require the same genetic pathways to regulate their development. To understand the roles of these genes in *M. sexta*, we used bioinformatics and molecular biological approaches to identify and characterize these genes. We identified a single putative *M. sexta* homolog of *Kirre/Rst,* as well as a single putative homolog of *Sns/Hbs.* A comparison of these putative genes across a variety of insects reveals that in most insects, a single paralog of *Kirre/Rst* and *Sns/Hbs* exists. Within Dipterans, however, a gene duplication event occurred that produced paralogous genes for both *Kirre* and *Rst*, as well as for both *Sns* and *Hbs*. This duplication occurred after the divergence of Nematoceran and Brachiceran lineages, as all mosquitos analyzed contain only a single paralog of *Kirre/Rst* and *Sns/Hbs.* This suggests that there was a concurrent duplication of both the ligand and receptor genes within the Dipteran lineage. The significance of this concurrent duplication is under investigation.

43. Investigation of Neuropeptide F as a Novel Insecticide Target

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Emerging and reemerging diseases, such as Chikungunya and Zika virus, threaten to become major public health concerns and more familiar diseases, like malaria and dengue fever, are infecting new populations due to lapses in vector control programs, development of insecticide resistance, human migration, and increasing vector habitat due to human activities. Developing new and innovative strategies to combat these tropical infectious diseases transmitted by mosquitoes is an enormous challenge facing global public health. While there have been efforts to control certain vector-borne diseases, these goals have proved frustratingly elusive and the incidence of many vector-borne infections is rising. Consequently, new insecticides that act by alternative pathways must be discovered. G-protein coupled receptors (GPCRs) are one group of potential novel insecticide targets since they are highly 'druggable' proteins. One GPCR of interest is the invertebrate neuropeptide F receptor and its associated ligand because it is known that the vertebrate ortholog affects food intake, digestion, metabolism, and reproduction. This study investigated neuropeptide F (NPF) GPCR and its associated ligand in *Aedes aegypti* and *Anopheles gambiae* mosquitoes. Three NPF receptors were identified in the *An. gambiae* genome and 8 in the *Ae. aegypti* genome. Of the 8 *Ae. aegypti* NPF receptors, AAEL10626 is the likely homologue of the *D. melanogaster* NPF receptor that is known to be involved in foraging. Alignment of AAEL10626 with the *An. gambiae* NPF receptor revealed an 84.6% amino acid identity and both receptors are most closely related to the vertebrate NPY_2 receptor family. Both the *Ae. aegypti* and *An. gambiae* NPF sequences encode for a 37 amino acid protein, exhibit a 76% identity at the amino acid level, and differ substantially from the NPY of human and boar. The *Ae. aegypti* AAEL10626 NPF receptor is most highly expressed in L1 larvae and adult males, but is also expressed in the adult female. Functional validation of *Ae. aegypti* AAEL10626 was completed, indicating that although AAEL10626 is annotated as a NPY receptor, it is a bona fide NPF receptor signaling through $G\alpha_i$. Knockdown of *Ae. aegypti* NPF and NFP receptor gene expression using double-stranded RNA silencing resulted in inhibition of both sugar and blood feeding of *Ae. aegypti* females, validating NPF receptor as an insecticide target.

44. Islands with moderate genetic differentiation and small effective population sizes of the malaria vector *Anopheles gambiae:* field sites for evaluating transgenic drive?

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Background: 68% of the estimated 429,000 deaths attributable to malaria in 2015 were African children < 5 years of age. This figure has reduced significantly in the last decade due to an expansion of control interventions, but the development of new technologies that complement existing strategies is still necessary to achieve malaria eradication. Much attention has recently been paid to the concept of genetically modified (GM) malaria vectors. Despite successful *in vivo* laboratory examples, a detailed, longitudinal population genetic study, which must first precede any proposed field trial, has yet to be undertaken systematically. This study aimed to explore the genetic structure of the malaria vector *Anopheles gambiae* Giles in six locations of northwestern Lake Victoria to assess whether any were potential candidates for a pilot field study release of GM mosquitoes.
Methods: Six populations of *An. gambiae* mosquitoes (N=594) were sampled from four island and two mainland sites. A subset (N=96) was selected for restriction-site associated DNA sequencing (RADseq) and the resulting single nucleotide polymorphism (SNP) markers were analyzed for population structure using principal components analysis (PCA), ADMIXTURE v.1.23, and F_{ST}. Estimations of effective population size (N_e) were calculated using the linkage disequilibrium method of NeEstimator v.2.01.

Results: After filtering the dataset based on genotype quality, we identified 5,175 SNPs across the genome. PCA showed that the inversion on chromosome 2La was highly influential on population structure, and as the inversion is correlated to ecological niche and seasonality, it was excluded from further analyses. In PCA of the colinear regions of the genome, individuals clustered in concordance with geographic origin, but with some overlap between sites. Genetic diversity between populations was relatively varied (median F_{ST} range: 0.0100 - 0.0903) with inter-island comparisons having the highest values. Estimates of N_e were generally low for these populations (124.2 - 1920.3). **Conclusions:** Genetic exchange is occurring in these populations (as evidenced by the varied F_{ST} range), which is a crucial element for the maintenance and dispersal of a transgenic drive system. That greater structure was observed in the island populations suggests that there is some limitation to migration between them. Smaller estimates of N_e indicate that an introduced transgene would be susceptible to genetic drift, but it must be paired with a robust effector mechanism to ensure that is driven to fixation instead of loss. Moderate levels of gene flow and small N_e, together with location and suitability for frequent monitoring, mean the Ssese Islands should be considered for further evaluation as candidate field sites for a GM mosquito pilot release.

45. Serotonin receptors as druggable targets

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Arthropods harbor causative agents of diseases pertinent to global health. *Aedes aegypti* and *Anopheles gambiae*, in particular, are primary vectors for the arbovirus and parasite responsible for dengue fever and malaria, respectively, afflicting high human morbidity and mortality worldwide. While potential vaccines have made significant strides through the development pipelines, control efforts have predominantly relied on the use of insecticides. Given the worldwide emergence of insecticidal resistance, however, identification of insecticides with alternative modes of action is of utmost importance. Biogenic amines and their G-protein coupled receptors (GPCRs) are ideal novel targets for investigation due to their role in neuronal function, influencing physiological processes and behavioral states. The serotonergic (5-HT) system has been documented to impact a wide array of processes involved in disease transmission, suggesting its potential to be a new avenue and mode of action target for insecticide development. Due to the availability of deep-sequencing data on various organisms and the presence of highly conserved regions of amino acids within the 5-HT GPCR family, sequence similarity and homology-based searches were performed to identify putative target genes in *Ae. aegypti* and *An. gambiae*. Target receptors were cloned and expressed in the human embryonic kidney (HEK293) cell line for functional and pharmacological characterization. To determine behavioral alterations, *in vivo* assays were conducted with chemical compounds reported to affect invertebrate 5-HT receptors. A strong antagonist of the 5-HT_2 subfamily demonstrates its ability to impact locomotion in a dose-dependent manner, as well as, blood-feeding behavior. This study seeks to address the strong need for novel insecticides by focusing on the discovery of chemistries disrupting the 5-HT GPCR gene target, and ultimately, the behavior of the mosquito.

46. Delivery of Gene BioTechnologies to Plants: Pathogen and Pest Control

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Treatment of oligonucleotides to plants for host delivered suppression of microbes and insect pests of citrus was successful. FANA_ASO, (2'-deoxy-2'-fluoro-D- arabinonucleic acid)_(antisense oligonucleotides-AUM LifeTech), designed to: Asian citrus psyllid; Citrus plant bacterial pathogen of citrus, *Candidatus* Liberibacter asiaticus (CLas), Psyllid endosymbionts. Treatments reduced CLas bacteria within infected citrus trees, reduced *Wolbachia* in cell cultures and insects. Suppression of endosymbionts resulted in increased psyllid mortality. Confocal microscopy and spectrophotometry detection supported systemic movement and cell entry. Bacteria titers were reduced in treated plants on average by 50%

(live/dead PMA bioassay) at three weeks post treatment. Adult insects showed delivery into hemolymph cells and organs. Results suggest significant potential for application of these products as a treatment to reduce pathogens of plants or animals, like Citrus greening disease. Supported in-part: NIFA, USDA, Citrus Greening award #2015-70016-23028; and NIFA, USDA, award #2015-10479.

47. Method for Detecting Binding Efficiencies of synthetic Oligonucleotides: Targeting Bacteria and Insects

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Expanding applications of gene-based targeting, biotechnology, in functional genomics, and the treatment of plants, animals, and microbes has synergized the need for new methods to measure binding efficiencies of these products to their genetic targets. The adaptation and innovative use of Cell–Penetrating-Peptide Morpholino, and antisense oligonucleotides depend on visual bound fluorophores, and confocal microscopy, or intensive chemical GC-MS analyses for confirmation of delivery. Visual confirmation is often plagued with problems of autofluorescence background, in plant and insect samples. Confirmation of the presence of labeled molecules can be detected using fluorescent plate readers, which provide a more rapid and cost effective analyses. However, neither confocal or plate reader, addresses the binding efficiency, binding competition, or detection of unlabeled oligonucleotides. Presented is a method using primers which flank or reside on the binding target as a means to quantify binding preference across known targets. The method can also be used in qPCR analyses to quantify bound to unbound oligonucleotides. When used as a prescreening method of molecules to validate binding efficiency, costs and time may be saved. Similar methods apply across technologies, like RNAi and CRISPR, Supported in-part: NIFA, USDA, Citrus Greening award #2015-70016-23028; and NIFA, USDA, award #2015-10479.

48. Genetics and genomics of host specificity in aphid parasitoids

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Differences in parasitism success among potential host species can provide strong selection for divergence and speciation in parasitic Hymenoptera. Here we report research on the genomics and genetics of host specificity in *Aphelinus* species. We have sequenced, assembled, and annotated the genomes and transcriptomes of >10 *Aphelinus* species. Using coding sequences, we developed a robust phylogeny, onto which we mapped parasitism of diverse species of aphids. For some aphid species, parasitism was phylogenetically conserved, with closely related parasitoids showing similar levels of parasitism. For other aphid species, parasitism diverged between closely related parasitoids, consistent with host-driven speciation. To explore the genetic architecture of differences in host specificity, we crossed and backcrossed *A. atriplicis*, which readily parasitizes *Diuraphis noxia*, with *A. certus*, which rarely parasitizes this aphid. Using genetic markers from reduced-representation genomic libraries, we mapped quantitative trait loci (QTL) affecting parasitism of *D. noxia*. We found eight QTL (six of which interacted in their effects) that explained 39% of the variation in parasitism *D. noxia* among backcross females. To help identify candidate genes, we compared the genomes and transcriptomes of these parasitoid species to find proteins that diverged in sequence or expression, and we tested whether these divergent loci mapped to QTL affecting parasitism of *D. noxia*. So far, we have found 15

divergent genes that mapped to parasitism QTL or significantly affected parasitism by themselves. Using RNA probes, we have shown expression of candidate gene g6935 in both ovipositor and antenna sensilla. These are among the first results on the genetic architecture of host specificity in parasitic wasps.

49. On the Origin of the W chromosome in Lepidoptera: Insights from the Z chromosome

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Moths and butterflies (Lepidoptera) together with their sister group caddisflies (Trichoptera) represent the most speciose taxon with WZ/ZZ sex chromosome system. Despite that, the origin of W chromosome in this group remains unclear. Since trichopterans and early diverging lepidopteran lineages share a Z0/ZZ constitution of sex chromosomes, it was generally assumed that the W chromosome evolved in the common ancestor of the clade Ditrysia comprising 98% of lepidopteran species and the nonditrysian family Tischeriidae. Here we provide evidence indicating that the W arose multiple times in Lepidoptera, most likely via a co-option of a B chromosome. Because the W chromosome itself cannot be directly studied due to a high level of degeneration, we have used various genetic and genomic approaches to examine the gene content of its evolutionary partner, the Z chromosome. We believe that the comparison of synteny of Z-linked genes in representatives of key lepidopteran families will help to finally resolve the evolutionary history of the W chromosome in Lepidoptera.

50. Systems biology resources for *Diaphorina citri*, a vector for the Citrusgreening disease

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We have designed a web portal with genomics and bioinformatics resources for the vector Asian citrus psyllid (ACP, *Diaphorina citri*), the host citrus (*C. clementina* and *C. sinensis*) and multiple pathogens including *Candidatus* Liberibacter asiaticus (CLas) for the citrus greening disease. We have used Biocyc Pathway Tools databases to model biochemical pathways within each organism that can be used to explore the entire disease complex. DiaphorinaCyc, the pathway database for *D. citri*, is available at http://ptools.citrusgreening.org/ where users can upload multi-omics data sets from transcriptomics and proteomics experiments to analyze pathways for differentially expressed genes. Psyllid Expression Network (PEN) with proteomics results for ACP from adult and nymph life stages, conditions and multiple hosts (*C. sinensis* and *C. medica*) is available at https://pen.sgn.cornell.edu. We have implemented JBrowse to provide the context for expression data and features annotated on the genomes. The interim ACP genome (v1.9) assembled from long reads is also available in JBrowse. We have assembled a *de novo* transcriptome for ACP (MCOT v1.1) which is included in the Blast databases. All tools like JBrowse, Biocyc, Blast and PEN connect to a central database containing gene models for citrus, ACP and multiple *Candidatus* Liberibacter pathogens.

The portal includes user-friendly manual curation tools to allow the research community to continuously improve this knowledge base as more experimental research is published. Bulk downloads are available for all genome and annotation datasets from the FTP site (ftp://ftp.citrusgreening.org). The portal can be accessed at https://citrusgreening.org/.

51. Genome assembly for experimental evolution in the pigeon louse *C. columbae*

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Coevolution between parasites and hosts is believed to be one of the primary drivers of adaptive evolution. Even so, the genomic basis of parasite-host coevolution is rarely fully understood. We have undertaken the use of *Columbicola*

columbae, the pigeon louse, as a model for understanding parasite-host coevolution. Pigeon lice are known to have strong correlations between their body colors and the body colors of their hosts, as well as their sizes and the sizes of gaps between host feathers, in which the lice reside. We have generated a thorough phylogeny for *C. columbae* using aTram targeted genome sequencing, and observe a great deal of variation in size and color that is correlated with the size and color of the host bird. We have experimentally evolved feral *C. columbae* to adapt to pigeons of different sizes and colors and can demonstrate repeated changes in size and color across replicate *C. columbae* populations. We are now using whole-genome long and short read sequencing to generate a highly contiguous genome assembly for further genomics. We plan to sequence pools of individuals from each of the experimentally evolved populations and statistically distinguish regions that have repeatedly evolved in replicate populations of similar treatments, thereby identifying the regions of the genome that underlie selection for defense against hosts.

52. Use of genome and transcriptome information for the development of male-only strains of the New World screwworm

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The New World screwworm, *Cochliomyia hominivorax,* is a devastating pest of livestock that was eradicated from North and Central America though repeated releases of sterilized males and females. The recent outbreak of screwworm in the Florida Keys and earlier in Libya in the 1980s, highlight the potential of screwworm as an invasive species. As the sterile insect technique is more efficient if only sterile male flies are released, we have developed transgenic male-only strains that carry conditional female lethal genes. With the initial strains, females died at the pupal stage on diet that lacked tetracycline. Several of the strains show production characteristics (e.g. egg hatch, pupal weight) and mating competitiveness comparable to the current mass reared strain. Selected strains are to be evaluated in open field tests and in large cages. Second generation systems designed to be lethal to females at the embryo stage have been developed. To build these systems we first identified transcripts that are highly expressed in embryos but at low levels at later stages. Promoters from these genes were identified from draft assemblies of blow fly genomes. A preliminary analysis of an assembled New World screwworm genome will be presented.

53. Metabolic communication: transporters play a key role in the tsetse-*Wigglesworthia* nutrient exchange

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Tsetse flies (Diptera: Glossinidae), the obligate vectors of African trypanosomes, feed exclusively on vertebrate blood. The most prevalent member of the tsetse microbiota, *Wigglesworthia*, is a Gammaproteobacterium harbored within the bacteriome organ, localized to the anterior midgut. The *Wigglesworthia* symbiont, through nutrient provisioning, enables the unique tsetse feeding ecology. Little is known regarding how endosymbionts coordinate activities and products with host biology.
An evident opportunity to examine this interspecies synchronization is assessing the metabolic activity occurring at the host-symbiont interface. Further, transporters are hypothesized to facilitate the coordination and systemic distribution of provisioned nutrients. Here, we characterize the metabolic activities of tsetse fly bacteriomes, isolated from Nguruman, Kenya, through Illumina-based transcriptomics. Special attention is given to both the identity and functional characterization of transporters within the tsetse and *Wigglesworthia* genomes.

Methods: Adult tsetse flies, *Glossina pallidipes,* were captured from Nguruman escarpment, Kenya, in March 2015. Bacteriomes were pooled, RNA isolated and cDNA synthesized for the construction of Illumina sequencing libraries. *G. pallidipes*-specific reads were identified based on the genome available at VectorBase with the remaining sequences then mapped to the *Wigglesworthia morsitans* (WGM) genome. In order to compare the expression of folate (B9) and thiamine (B1) transporters across tsetse species, *G. morsitans* and *G. fuscipes* bacteriomes were dissected and RNA isolated, with the corresponding orthologs amplified via RT-PCR. TRANSPORTDB 2.0 identified the predicted cytoplasmic transport proteins within the *Wigglesworthia* genome. Additionally, TRANSPORTDB 2.0 was also used to

retrieve transporters present in the *Drosophila melanogaster* genome, with *G. morsitans* orthologs subsequently found though FlyBase and the *G. pallidipes* orthologs through VectorBase.

Results: Within adult bacteriomes, and highly mirroring the *Wigglesworthia* symbiont profile, there was a universal enrichment of expressed tsetse genes with GO terms associated with metabolism and transport. Out of the 55 transporters reported for the *Wigglesworthia* genome, 38 (69%) were found transcribed with 9 (16%) of these having TPM values 1.5 fold higher than the average within the bacteriomes of field flies. Robust expression of transporters involved in multidrug efflux, glutamine and cystine ABC-binding and ATP synthases were identified. Accordingly, multiple expressed tsetse loci were associated with roles in conveying substrates into and out of tsetse bacteriocytes with a number relevant to vitamin transport including thiamine, riboflavin (B2), folate and a multivitamin transporter. We confirmed that reduced folate carrier and thiamine transporter were both expressed in the bacteriome and head tissue of adults of two other tsetse species, *G. morsitans* and *G. fuscipes*.

Future: The knowledge of overrepresented metabolic activities within bacteriomes and the identification of transporters dedicated to shuffling substrates between tsetse and its symbionts could prove key in translational epidemiology combating African trypanosomiasis. The tsetse microbiota may ultimately be used for innovative symbiont-based control mechanisms.

Keywords: symbiont, tsetse, metabolic complementarity, translational epidemiology, folate, transporter, transcriptome, sleeping sickness, microbiota, bacteriome

54. Genetic mechanism underlying adaptive variation of bumblebee mimetic coloration

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There are rich examples of adaptive variations as results of natural selection,. but we still have a long way to go in understanding how Yet little is known how selection sculptures the most basic level of life, the genome, to promote phenotypic variations and diversification. To address this question, we study are studying the genetic mechanisms underlying bumblebee color pattern polymorphism, an adaptive phenotypic radiationvariation driven by Müullerian mimicry. Using advanced bioinformatics tools such as GWAS, we have identified genetic changes potentially involveddriving with a red/black abdominal dorsal hair color polymorphism of in the bumble bee *Bombus melanopygus.* Using molecular developmental genetic tools such as RNA insitu and qRT-PCR, we are investigating how these genetic changes alter gene regulation to create distinct phenotypes. Meanwhile, we are performing comparative study with With this adaptive locus in hand, we are comparing whether another red/black polymorphic polymorphisms in comimetic species, *B. bifarius* to understand if same or differentutilize the same genetic mechanisms are involved in the parallel color pattern evolutionto attain parallel color variation. Our study will provideprovides new insights into how changes in gene regulation can promotethe genome drive and promote adaptive diversification.

55. Retention of duplicated long-wavelength opsins in mosquito lineages by positive selection and differential expression.

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Opsins are light and visual sensitive receptors. Insects typically possess opsins that are stimulated by ultraviolet, short and long wavelength (LW) radiation, but other types have also been described. Six putative LW-sensitive opsins predicted in the yellow fever mosquito, *Aedes aegypti* and malaria mosquito, *Anopheles gambiae,* and eight in the southern house mosquito, *Culex quinquefasciatus*, suggest gene expansion in the Family Culicidae (mosquitoes) relative to other insects. Here we report the first detailed molecular and evolutionary analyses of LW opsins in three

mosquito vectors, using published genomes, transcriptomes and proteomes, with a goal to understand the molecular basis of opsin-mediated visual processes that could be exploited for mosquito control. The transmembrane domains of the mosquito LW opsins share between 60 to 100% amino acid identity. Time of divergence estimates suggest that the mosquito LW opsins originated from 18 or 19 duplication events between 166.9/197.5 to 1.07/0.94 million years ago (MY) and that these likely occurred following the predicted divergence of the lineages Anophelinae and Culicinae 145–226 MY. Nine amino acid residues in the LW opsins were identified under positive selection. Of these, eight amino acids occur in the N and C termini and are shared among all three species, and one residue in TMIII was unique to culicine species. Alignment of 5′ non-coding regions revealed potential Conserved Non-coding Sequences (CNS) and transcription factor binding sites (TFBS) in seven pairs of LW opsin paralogs. Our analyses suggest opsin gene duplication and residues possibly associated with spectral tuning of LW-sensitive photoreceptors. We explore two mechanisms - positive selection and differential expression mediated by regulatory units in CNS – that may have contributed to the retention of LW opsin genes in Culicinae and Anophelinae. We discuss the evolution of mosquito LW opsins in the context of major Earth events and possible adaptation of mosquitoes to LW-dominated photo environments, and implications for mosquito control strategies based on disrupting vision-mediated behaviors.

56. Genome assembly of the Luna Moth, *Actias luna* (Lepidoptera: Saturniidae)

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The Luna Moth is a member of the lepidopteran family Saturniidae, a diverse group of moths that belong to the superfamily Bombycoidea. These moths are characteristically sexually dimorphic, large in size and brightly colored with some species having well-developed wing eyespots. Saturniids are also one of the two primary silk-producing families within the Bombycoidea. However, many of the silk-producing genes identified in closely related taxa have yet to be identified within the family. Here, we report a preliminary PacBio genome assembly and annotation for the luna moth, *Actias luna*, and ongoing associated RNASeq work.

57. Gene expression profiles of gene associated with silk production in Dysdera spiders

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The molecular basis of spider silk production is of broad interest because of its possible mechanical applications. For instance, dragline silk, which is produced in the major ampullate gland of certain spiders, has been found to be tougher than nylon and Kevlar®. However, even though there is research on the mechanical and structural properties of spider silk, the gene expression and regulation responsible for spider silk production remains largely unexplored. In this project, we are trying to identify the genes that regulate spider silk production by analyzing 8 RNAseq libraries from silk glands of male and female Dysdera spiders. Using a reference transcriptome, a differential expression analysis was performed to identify statistically relevant expressed genes. The programs BowTie2 and TopHat were used to perform alignment to a reference transcriptome. Two different pipelines were used to perform differential expression analysis: the first is using the tuxedo tool suite of programs (CuffLinks, CuffMerge, CuffCompare, CuffDiff, and cummeRbund) and the second is using HTSeq and DESeq. These two most widely used pipelines in RNAseq analysis statistically determine significant differentially expressed genes.

58. Using novel, low-cost sequencing technologies to increase genomic resources for agricultural pests

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Global shipping and trade practices greatly facilitate the introduction of insect pests into new host ranges. Once established, these invaders can have detrimental impacts on local ecosystems, agricultural production, and even food processing/storage facilities, which can provide optimal sources of nutrition and environmental conditions that enable rapid population growth. Often times, not much is known about the biology of these introduced pests, but it is essential to devise rapid and effective control measures to eliminate and prevent them from spreading beyond the initial site of introduction. Recent insect genome sequencing efforts have greatly expanded our understanding of the metabolic and physiology capacities of emerging invasive pests and have also lead to the identification of genetic factors that contribute to host range, tolerance to biotic stresses, and resistance to various insecticides, all of which can greatly expedite management decisions. However, the inability to obtain sufficient concentrations of high-quality DNA has impeded the rapid development of genome assemblies for many economically devastating agricultural pests. Using 10X Chromium libraries, we have obtained high quality draft assemblies of three major aphid pests of bioenergy grasses (yellow sugar cane aphid, sugar cane aphid, and greenbug) with total assembly lengths that exceeded 90% of the estimated genome sizes and had contig N50s>100kb and scaffold N50s>2 Mb. The DNA libraries were created from pools of multiple clonal individuals, demonstrating that high quality *de novo* genome assemblies can be created for aphids using10X Chromium libraries. In addition, we are also applying these library and assembly techniques to stored product pests belonging to five different insect families, including two globally invasive species (khapra beetle; *Trogoderma graminum* and larger grain borer; *Prostephanus truncatus*). In these cases, DNA isolated from single individuals was used to prepare Chromium libraries in order to circumvent inbreeding, which can be time consuming and difficult for certain insect species, and pooling multiple individuals, which can cause assembly breaks due to allelic differences between individuals and heterozygosity. The 10X assemblies will ultimately be coupled with high density linkage maps and Oxford Nanopore sequencing to generate higher order scaffolds. Once completed, these genomes will provide us with platforms to study the genetic mechanisms of pesticide resistance, tolerance to stresses, and ability to perceive and respond to food odors in a variety of different insect pests.

59. Transcriptome assembly and differential expression analysis of western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in response to Bt intoxication

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The western corn rootworm (WCR) is a non-model insect species that causes severe damage to maize. It is a highly adaptive species and has evolved resistance to several Bt transgenic maize lines, which have been widely adopted as a management strategy in the U.S Corn Belt. The Bt resistance mechanism in WCR has not been well studied. We used the Illumina HiSeq 2000 system to sequence WCR larvae and midgut transcriptomes. Three *de novo* assembly approaches were evaluated and optimized using larval data. We obtained 204,842 and 218,683 contigs respectively from whole larvae and midgut tissue. We used edgeR-robust to perform differential expression analysis among colonies and feeding treatments. The transcriptome profile of susceptible WCR changed dramatically in response to Bt intoxication, while that of resistant WCR exhibited less change. The expression patterns and annotation of differentially expressed genes may provide the insight into the mode of action of Bt toxin and the potential resistance mechanism of WCR against Bt proteins.

60. A comprehensive bioinformatic guideline for conducting Genome Wide Association Studies (GWAS) in haploid non-model organisms

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Genome-wide association analysis is a widely-used technique to identify the candidate regions associated with phenotypes, with greatest use in identifying markers underlying human diseases and adaptive traits in model organisms. However, appropriate genomic tools/resources are not available/incomplete in most of the non-model species and these tools are not designed to deal with ploidy issue of haploid/polyploid datasets. Here, we provide a comprehensive pipeline to conduct GWAS in non-model haploid organisms which cover experimental design, genome sequencing, alignment, variant calling, genotype-phenotype association analysis and interpretation/visualization of the results. This pipeline, includes parameter adjustments, custom scripts for batch processing/integration and utilization of introduced features of existing *de facto* standard tools in bioinformatics/human genomics (such as BWA, VCFtools, PLINK) to accommodate non-diploid organism datasets. This pipeline has been utilized to conduct GWAS in a bumblebee dataset (20 haploid whole genome sequencing of paired-end libraries) which aimed to identify the genomic basis of red-black colour pattern transition (a case-control trait) in the mimetic species B*ombus melanopygus.* Our analysis revealed the candidate locus driving abdominal hair color polymorphism in the mimetic species *Bombus melanopygus* to a cis-regulatory region of the abdominal Hox genes. Our guideline can also be used with slight modification to identify the genomic basis of quantitative traits.

61. Islands with moderate genetic differentiation and small effective population sizes of the malaria vector *Anopheles gambiae:* field sites for evaluating transgenic drive?

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Background: 68% of the estimated 429,000 deaths attributable to malaria in 2015 were African children < 5 years of age. This figure has reduced significantly in the last decade due to an expansion of control interventions, but the development of new technologies that complement existing strategies is still necessary to achieve malaria eradication. Much attention has recently been paid to the concept of genetically modified (GM) malaria vectors. Despite successful *in vivo* laboratory examples, a detailed, longitudinal population genetic study, which must first precede any proposed field trial, has yet to be undertaken systematically. This study aimed to explore the genetic structure of the malaria vector *Anopheles gambiae* Giles in six locations of northwestern Lake Victoria to assess whether any were potential candidates for a pilot field study release of GM mosquitoes.
Methods: Six populations of *An. gambiae* mosquitoes (N=594) were sampled from four island and two mainland sites. A subset (N=96) was selected for restriction-site associated DNA sequencing (RADseq) and the resulting single nucleotide polymorphism (SNP) markers were analyzed for population structure using principal components analysis (PCA), ADMIXTURE v.1.23, and F_{ST}. Estimations of effective population size (N_e) were calculated using the linkage disequilibrium method of NeEstimator v.2.01.
Results: After filtering the dataset based on genotype quality, we identified 5,175 SNPs across the genome. PCA showed that the inversion on chromosome 2La was highly influential on population structure, and as the inversion is correlated to ecological niche and seasonality, it was excluded from further analyses. In PCA of the colinear regions of the genome, individuals clustered in concordance with geographic origin, but with some overlap between sites. Genetic diversity between populations was relatively varied (median F_{ST} range: 0.0100 - 0.0903) with inter-island comparisons having the highest values. Estimates of N_e were generally low for these populations (124.2 - 1920.3).

Conclusions: Genetic exchange is occurring in these populations (as evidenced by the varied F_{ST} range), which is a crucial element for the maintenance and dispersal of a transgenic drive system. That greater structure was observed in the island populations suggests that there is some limitation to migration between them. Smaller estimates of N_e indicate that an introduced transgene would be susceptible to genetic drift, but it must be paired with a robust effector mechanism to ensure that is driven to fixation instead of loss. Moderate levels of gene flow and small N_e, together with location and suitability for frequent monitoring, mean the Ssese Islands should be considered for further evaluation as candidate field sites for a GM mosquito pilot release.

62. Targeted next-generation sequencing of detoxification genes in *Culex pipiens* complex mosquitoes: Discovery of SNPs and copy number variants associated with insecticide resistance

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Insecticide resistance in vector mosquitoes can increase the frequency with which insecticides are applied, require more insecticide to be used and reduce the ability of vector control districts to reduce mosquito populations during a disease outbreak. Using Life Technology's Ampliseq process, we developed a panel of 115 genes known or hypothesized to be involved in insecticide resistance. The panel is run on an Ion Torrent PGM sequencer. Individual families of *Culex quinquefasciatus* were tested for resistance to Permethrin and Malathion using a bottle bioassay. Data were analyzed by comparing resistant to susceptible individuals (n= 106 unrelated individuals). We conducted analyses to discover two kinds of genetic changes associated with resistance: SNPs (single nucleotide polymorphisms) and CNVs (copy number variants). Genes with statistically significant SNPs included the gene associated with kdr (knock down resistance), as well as esterases, glutathione S-transferases and cytochrome P450s. Similarly, genes shown to exist in increased copy numbers included two esterase genes known to be duplicated in individuals showing metabolic resistance, as well as the gene associated with kdr, and several cytochrome P450 genes. These results will be used to develop PCR based assays to better characterize resistance in field-collected mosquitoes. We are also using the panel to sequence *Culex pipiens* from the U.S., and *Culex pipiens* form molestus from the U.S. and several locations internationally to 1) develop an updated assay to distinguish *Cx. pipiens* from *Cx. quinquefasciatus* in the U.S. and 2) conduct a phylogenetic analysis of U.S. and international specimens of *Cx. pipiens* form molestus.

63. Investigation of Neuropeptide F as a Novel Insecticide Target

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Emerging and reemerging diseases, such as Chikungunya and Zika virus, threaten to become major public health concerns and more familiar diseases, like malaria and dengue fever, are infecting new populations due to lapses in vector control programs, development of insecticide resistance, human migration, and increasing vector habitat due to human activities. Developing new and innovative strategies to combat these tropical infectious diseases transmitted by mosquitoes is an enormous challenge facing global public health. While there have been efforts to control certain vector-borne diseases, these goals have proved frustratingly elusive and the incidence of many vector-borne infections is rising. Consequently, new insecticides that act by alternative pathways must be discovered. G-protein coupled receptors (GPCRs) are one group of potential novel insecticide targets since they are highly 'druggable' proteins. One GPCR of interest is the invertebrate neuropeptide F receptor and its associated ligand because it is known that the vertebrate ortholog affects food intake, digestion, metabolism, and reproduction. This study investigated neuropeptide F (NPF) GPCR and its associated ligand in *Aedes aegypti* and *Anopheles gambiae* mosquitoes. Three NPF receptors were identified in the *An. gambiae* genome and 8 in the *Ae. aegypti* genome. Of the 8 *Ae. aegypti* NPF receptors, AAEL10626 is the likely homologue of the *D. melanogaster* NPF receptor that is known to be involved in foraging. Alignment of AAEL10626 with the *An. gambiae* NPF receptor revealed an 84.6% amino acid identity and both receptors are most closely related to the vertebrate NPY_2 receptor family. Both the *Ae. aegypti* and *An. gambiae* NPF sequences encode for a 37 amino acid protein, exhibit a 76% identity at the amino acid level, and differ substantially from the NPY of human and boar. The *Ae. aegypti* AAEL10626 NPF receptor is most highly expressed in L1 larvae and adult males, but is also expressed in the adult female. Functional validation of *Ae. aegypti* AAEL10626 was completed, indicating that although AAEL10626 is annotated as a NPY receptor, it is a bona fide

NPF receptor signaling through $G\alpha_i$. Knockdown of *Ae. aegypti* NPF and NFP receptor gene expression using double-stranded RNA silencing resulted in inhibition of both sugar and blood feeding of *Ae. aegypti* females, validating NPF receptor as an insecticide target.

64. GPCR Targeted Insecticide Design for Control of Vector Mosquitoes Transmitting Dengue and Zika

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Zika and Dengue are infectious diseases that are caused by the vector mosquito Aedes aegypti. The spread of the diseases has increased the need for containment and eradication.Past development of insecticide drugs, including permethrin and DDT are starting to become unsatisfactory due to the increased resistance of disease carrying vectors. New techniques of vector control and disease prevention look to G Protein Coupled Receptors (GPCR's) as insecticide targets. GPCRs play a huge role in transducing extracellular stimuli into intracellular stimuli that are part of signaling pathways affecting many physiological events in mosquitoes. GPCRs respond to ligands that are easily manipulated, essentially "easily druggable", meaning that it is easy to develop compounds that can affect the receptors. Octopamine receptors is one of the identified GPCRs that bind to octopamine which is a neuromodulator involved in multiple cellular pathways. A growing number of studies have suggested that octopamine plays a prominent role in modulating physiological and behavioral processes in invertebrates. The octopamine receptor appears to not be present in vertebrates, which could mean that they present as a suitable insecticide target. Amitraz is a current effective acarid pesticide that targets octopamine receptors and was thought to have the same effect on mosquitoes. This was not observed after experimentation with adult Aedes aegypti, but the octopamine receptor that the insecticide targeted was still a basis for further research. Compounds, like formamide insecticides, LG-8, related to Amitraz could serve as ligands that can act as an agonist for octopamine receptors. Formamide insecticides act as an agonist for octopamine receptors, activating the receptor better than octopamine itself. To determine the effect of LG-8 on mortality we conducted a variation of the WHO susceptibility test. The ability of LG-8 to kill in a dose dependent manner shows the need for further investigation of the compound and its potential use as a new insecticide on the market. Future studies will continue to focus on LG-8 and modifying it to optimize its effectiveness while minimizing its toxicity to humans and the environment.

65. The making of a pest: Insights from the evolution of chemosensory receptor families in a pestiferous and invasive fly, *Drosophila suzukii*

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Drosophila suzukii differs from other *melanogaster* group members in their proclivity for ovipositing in fresh fruit rather than in fermenting fruits. Olfaction and gustation play a critical role during insect niche formation, and these senses are largely mediated by two important receptor families: olfactory and gustatory receptors (*Ors* and *Grs*). Here, we manually annotated the *Ors* and *Grs* in *D. suzukii* and two close relatives, *D. biarmipes* and *D. takahashii*, and compared these repertoires to those in six other *melanogaster* group drosophilids to identify candidate chemoreceptors associated with *D. suzukii*'s unusual niche utilization. We annotated a total of 71 *Or* genes in *D. suzukii*, with nine of those being pseudogenes (12.7 %). Alternative splicing of two genes brings the total to 62 genes encoding 66 *Ors*. Duplications of *Or23a* and *Or67a* expanded *D. suzukii*'s *Or* repertoire, while pseudogenization of *Or74a*, *Or85a,* and *Or98b* reduced the number of functional *Ors* to roughly the same as other annotated species in the *melanogaster* group. Seventy-one intact *Gr* genes and three pseudogenes were annotated in *D. suzukii*. Alternative splicing in three genes brings the total number of *Grs* to 81. We identified signatures of positive selection in two *Ors* and three *Grs* at nodes leading to *D. suzukii*, while one copy in a highly expanded *Or* lineage, *Or67a,* showed signs of positive selection in *D. suzukii*. Our analysis of chemoreceptor repertoires among nine *Drosophila spp*. revealed several candidate receptors associated with the adaptation of *D. suzukii* to its unique ecological niche.

2017 10th Arthropod Genomics Symposium

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INDEX

NOTES

NOTES

NOTES

NOTES

Campus Dining

Hours for June 5 – 18 (subject to change).

Dining Halls

North Dining Hall
closed

South Dining Hall
Breakfast: 6:30am - 9:00am
Lunch: 11:00am - 1:30pm
Dinner: 4:30pm - 7:30pm

Huddle Food Court

Huddle Mart
June 5 - 9 7:30am - 6:00pm
June 10 -11 closed
June 12 -16 7:30am - 6:00pm
June 17 closed
June 18 8:00am - 8:00pm

Starbucks
June 5 - 9 7:30am - 5:00pm
June 10 - 11 closed
June 12 - 16 7:30am - 5:00pm
June 17 closed
June 18 8:00am - 4:00pm

Smashburger
June 5 - 9 10:30am - 3:00pm
June 10 - 11 closed
June 12 - 16 10:30am - 3:00pm
June 17 - 18 closed

Subway
June 5 - 9 7:30am - 5:00pm
June 10 - 11 closed
June 12 - 16 7:30am - 5:00pm
June 17 closed
June 18 8:00am - 7:00pm

Taco Bell/Pizza Hut
June 5 - 18 closed

Express Stores

Au Bon Pain
(Hesburgh Library)
Monday - Friday 7:00am - 5:00pm
Saturday - Sunday closed

Au Bon Pain Express
(Hesburgh Center)
Monday - Friday 10:00am - 3:00pm
Saturday - Sunday closed

Cafe de Grasta
Monday - Friday 7:00am - 3:00pm
Saturday - Sunday closed

Decio Cafe
Monday - Friday 7:30am - 3:00pm
Saturday - Sunday closed

a la Descartes Cafe Commons Cafe Poche Crossings Kitz Kafé, Waddick's
closed

Restaurants

Reckers
Open 8:00am - 5:00pm Daily

Legends
Monday - Saturday 11:00am - 10:00pm
Sunday11:00am - 9:00pm