2022 Arthropod Genomics Symposium

June 9 – June 11, 2022

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Arthropod Genomics Center
Kansas State University

ECK INSTITUTE FOR
GLOBAL
HEALTH

2022 Arthropod Genomics Symposium
June 9 – 11, 2022

Schedule of Events

Thursday, June 9
Smith Ballroom Concourse, The Morris Inn

5:30 – 7:00pm Registration

6:00 – 7:30pm Welcome
Buffet Dinner

7:30 – 7:45pm Opening Remarks

7:45 – 8:45pm Keynote Lecture (Virtual) – Carolina Barrillas-Mury, LMVR, NIAID, NIH *"Mosquito immune evasion and the dispersal of ancestral Plasmodium falciparum and globalization of malaria"*

8:45 – 10:30pm Welcome Reception

Friday, June 10
Smith Ballroom, The Morris Inn

7:00 – 8:00am Poster set up (Posters 1-31)
Smith Ballroom Salon A

7:00 – 8:30 Continental Breakfast

8:00 – 8:45 Pan-Pacific Session
Session Chair: Mike Pfrender, University of Notre Dame

Fei Li, Institute of Insect Science, China (Virtual) – "*Genomes of rece insect pests and their parasitic wasps*"

Yiyuan Li, Institute of Plant Virology, China (Virtual) – "*Species divergence in gut-restricted bacteria of social bees*"

8:45 – 10:45am i5K/Emerging Genomes Session
Session Chair: Josh Benoit, University of Cincinnati

i5K Leadership, Anna Childers & Robert Waterhouse – "*The Future of i5K*"

Robert Waterhouse, University of Lausanne and the Swiss Institute of Bioinformatics – "*i5k in the landscape of global, regional, and national BioGenome Initiatives*"

Clement Goubert, McGill University (Virtual) – "*Sitphilus oryzae is dominated by transposable elements and shades light on the evolution of endosymbiosis in a major crop pest*"

Robert King, Rothamsted Research (Virtual) – "*The Pest Genomics Initiative*"

Scott Emrich, University of Tennessee – "*Targeted sequencing detects candidate resistance alleles to Bacillu thuringiensis insecticidal proteins in invasive Spodoptera frugiperda (Lepidoptera: Noctuidae)*"

10:45 – 11:15am Break

11:15 – 12:45pm Structural Evolution Session
Session Chair: Anna Childers, USDA-ARS

Emily Setton, University of Wisconsin-Madison – *"Using bioinformatics and RNA interference to investigate the genetic architecture of body plan patterning in spiders"*

Yasir Ahmed-Braimah, Syracuse University – *"The evolution of interspecific reproductive incompatibilities in Drosophila"*

Jiangtao Liang, Virginia Polytechnic and State University – *"Discovery of large chromosomal inversions in the genome of Aedes aegypti across the tropics"*

Andrew Mongue, University of Edinburgh (Virtual) – *"Paternal genome elimination creates contrasting evolutionary patterns in male and female citrus mealybugs"*

12:45 – 2:00pm Lunch on own
i5K Luncheon Discussion, Pre-registered, Private Dining Room

2:00 – 3:30pm Dessert, coffee, and Poster Session I
Posters, Smith Ballroom Salon A

3:30 – 5:00pm Vector Genomics Session
Session Chair: Molly Duman Scheel, Indiana University School of Medicine – South Bend

Noah Rose, Princeton University – "*Genomic basis of transitions between human-specialist and generalist ecology in Ae. aegypti's native range* "

Nic Buchon, Cornell University – "*A transcriptomic approach to mosquito anatomy, physiology and immunity*"

David Stanford-Beale, Purdue University – "*Thrips (Thysanoptera: Paraneoptera) have an ancient relationship with their vectored tospoviruses: a genomic approach to predicting hidden vectors of disease*"

Pauline Karega, University of Nairobi (Virtual) – "*Evolutionary characterization of innate immune related genes in six Glossina species"*

5:00 – 6:00pm Remove posters (Posters 1-31)

6:00 – 9:00pm Buffet Dinner, Dahnke Ballroom Notre Dame Stadium
African Genomics Session
Session Chair: Sam Rund, University of Notre Dame

Elijah Juma, Pan African Mosquito Control Association, Africa – "*The PAMCA Vector Genomics Suveillance Program: An overview"*

Sandrine Nsango, University of Douala, Cameroon – "*Malaria vector surveillance and population genetics characterization in Central Africa"*

Eric Ochomo, Kenya Medical Research Institute, Kenya – "*Updates on ongoing evaluation of vector control interventions and operational research around vector surveillance"*

Yaw Afrane, University of Ghana Medical School, Ghana – "Genetic diversity and population structure of *Anopheles funestus* in western Kenya"

Saturday, June 11
Smith Ballroom, The Morris Inn

7:00 – 8:00am Poster set up (Posters 32-63)
Smith Ballroom Salon A

7:00 – 8:30 Continental Breakfast

8:00 – 8:45 Pan-Pacific Session
Session Chair: Mike Pfrender, University of Notre Dame

Eddy Dowle, University of Otago, Dunedin, New Zealand (Virtual) – "*Genomic hijacking-how parasitic worms manipulate their insect hosts"*

Xin Zhou, China Agricultural University, China (Virtual) – "*What forces shape you: The genomics to the dark side"*

8:45 – 10:15am Arthropod Omics Session
Session Chair: Gloria Giraldo-Calderon, Universidad Icesi, Columbia

Marie Fablet, Claude Bernard University, France – "*Viral infection impacts transposable element transcript amounts in Drosophila"*

Lindsey Mack, University of California-Davis – "*Analysis of the Aedes aegypti gonotrophic cycle using synchrotron x-ray microCT*"

Martina Dalikova, The University of Kansas – "*Assembly and characterization of W chromosome in monarch butterfly (Danaus plexippus)*"

Hojun Song, Texas A&M University – "*Schistocerca (Orthoptera: Acrididae) as a model clade for studying phenotypic pasticity from a genomic perspective*"

10:15 – 10:45am Break

10:45 – 12:15pm Functional Genomics Session
Session Chair: Scott Emrich, University of Tennessee

Dana Shaw, Washington State University – "*Stress responses elicit a noncanonical mode of IMD pathway activation in ticks*"

Monika Gulia-Nuss, University of Nevada, Reno – "*From the genome to the phenome: tools to understand the basic biology of ticks*"

Hasiba Asma, University at Buffalo – "*Annotating insect regulatory genomes*"

Anita Lerch, University of Notre Dame – "*Validation of adult mosquito population size estimation with close-kin mark-recapture (CKMR) and amplicon sequencing*"

12:15 – 2:00pm Lunch on own

2:00 – 3:30pm Dessert, coffee, and Poster Session II
Posters, Smith Ballroom Salon A

3:30 – 5:00pm Microbiome Session
Session Chair: Monica Poelchau, USDA-ARS

Irene Garcia Newton (Virtual) – "*Mi casa es su casa: how an intracellular symbiont manipulates host biology*"

Katherine Brown, University of Cambridge – "*Identification of viral transcripts in RNA-seq datasets from bees, wasps, mites and ants*"

Allison Hansen, University of California, Riverside (Virtual) – *"Chromosomal-level assembly of Bacericera cockerelli reveals rampant gene family expansions and duplications of horizontally transferred genes that impact insect-microbe-plant-interactions"*

Bethanie Pelloquin, London School of Hygiene and Tropical Medicine – *"Overabundance of Asaia and Serratia bacteria is associated with deltamethrin insecticide susceptibility in Anopheles coluzzii from Agboville, Cote d'Ivoire""*

5:00 – 6:00pm Remove posters

6:00 – 9:00pm Dinner, Pre-registered only
Foley's, Notre Dame Stadium

Abstract Table of Contents

Platform/Oral Presentations

Posters

I5k/Emerging Genomes

Structural Evolution

Vector Genomics

9. ***Evolutionary characterization of innate immune related genes in six Glossina species*** - Karega Pauline (Virtual)

10. ***Thrips (Thysanoptera: Paraneoptera) have an ancient relationship with their vectored tospoviruses: a genomic approach to predicting hidden vectors of disease*** - David A. C. Stanford-Beale

11. ***Validation of adult mosquito population size estimation with close-kin mark-recapture (CKMR) and amplicon sequencing*** - Anita Lerch

12. ***Population genomics of "Apple Proliferation" disease transmissibility by apple psyllids and their endosymbionts*** - James M. Howie (Virtual)

13. ***Spiroplasma-induced changes in gene expression in the Glossina fuscipes fuscipes midgut*** - Erick Awuoche

14. ***Populations of Aedes aegypti in Colombia Shed Light on Gene Flow Patterns in the Americas*** - R. Rebecca Love (Virtual)

15. ***Gene regulation by mating depends on sugar water and tissue type in female Aedes aegypti*** - Ferdinand Nanfack-Minkeu

16. ***Identification and trends of tRNA genes in Diptera genomes*** - Melissa Kelley

17. ***Population genetic structure and diversity for Anopheles funestus based on mitochondrial DNA markers (mtDNA-COI and mtDNA-COII) in western Kenya*** - Isaiah Debrah (Virtual)

18. ***Evolutionary profile of knockdown resistance (kdr) mutation in the malaria vectors Anopheles gambiae and Anopheles coluzzii malaria vectors in the mountainous plains of Cameroon*** - Nathalie Amvongo-Adjia (Virtual)

19. ***Molecular evolution and epigenetics of Thysanoptera derived from the Frankliniella fusca genome assembly*** - Michael A. Catto

20. ***A chromosome-level assembly of the "Rockefeller" Aedes aegypti mosquito strain powers investigation of the evolution of pesticide resistance in a vector of human and livestock disease*** - Cera Fisher

21. ***Curation and phylogenetic analysis of Lutzomyia longipalpis and Phlebotomus papatasi Cytochrome P450s*** - Jason Charamis

22. ***Expanding Ensembl Metazoa arthropods*** - Jorge Alvarez-Jarreta (Virtual)

23. ***Differential gene expression in Culex pipiens mosquitoes exposed to different avian Plasmodium lineages.*** - Marta Garrigos (Virtual)

24. ***Evaluation of the immunogenic properties of Trypanosoma cruzi consensus enolase using a bioinformatics approach*** - Alejandro Díaz-Hernández

25. ***Gene expression and metabolite changes in the American dog tick during pesticide exposure*** - Souvik Chakraborty

26. ***Molecular characterization and transcriptomic analysis of leishmaniasis vectors in Sudan*** - Arwa Elaagip

27. ***Genetic diversity of genes involved into Anopheles gambiae s.l' fertility and vector competency in Sub-Saharan Africa*** - Fatoumata Seck (Virtual)

Arthropod Omics

28. ***Assembly and characterization of W chromosome in monarch butterfly (Danaus plexippus)*** - Martina Dalikova

29. ***Genome size evolution in the diverse insect order Trichoptera*** - Jacqueline Heckenhauer (Virtual)

30. ***Investigating the unusual chromatin organization and other unique biological phenomena, in the mealybug Maconellicoccus hirsutus using multi-omics approach*** - Surbhi Kohli

31. ***The evolution of insect visual opsin genes with specific consideration of the influence of ocelli and life history traits*** - Quentin Guignard (Virtual)

32. ***A chromosome-level genome assembly of Daphnia pulex*** - Zhiqiang Ye

33. ***A multi-omics approach reveals contrasting population-specific genome-wide epigenetic profiles across latitude-altitude range extremes in the bumble bee B. vosnesenskii*** - Sarthok Rasique Rahman

34. ***Molecular evolution of Cytochrome P450s in Tephritidae*** - Jason Charamis

35. ***The Glassy-winged Sharpshooter genome sheds light on the molecular basis of brochosomes*** - Zheng Li

36. ***HymenopteraMine: Improved homology and gene ontology enrichment analyses*** - Deborah A Triant

37. ***Chromosomal-level reference genome of the moth Heortia vitessoides (Lepidoptera: Crambidae), a major pest of agarwood-producing trees*** - Sean T.S. Law (Virtual)

38. ***Horseshoe crab genomes reveal the evolution of genes and microRNAs after three rounds of whole genome duplication*** - Hing-man Au (Virtual)

39. ***Genome and sex-biased transcriptomic response to temperature change of common yellow butterfly Eurema hecabe (Family Pieridae)*** - Ivy H.T. Lee (Virtual)

40. ***Dehydration and viral infection yield similar stress that deplete glycogen and increase feeding in the Western Flower Thrips, Frankliniella occidentalis*** - Samuel T. Bailey

41. ***Rhipicephalus microplus VDAC, a novel vaccine candidate, contains conserved B-cell epitopes that induce antibodies in immunized cattle*** - Juan Mosqueda

42. ***Evolution of non-insect/crustacean arthropods: from genomics to sesquiterpenoid endocrinology*** - Wai Lok So (Virtual)

43. ***Metabarcoding and population genomics to assess usage of alternate habitats by Rhyzopertha dominica (Coleoptera: Bostrichidae)*** - Erin D. Scully

Functional Genomics

44. ***Annotating insect regulatory genomes*** - Hasiba Asma

45. ***How do sizes of host-use gene families differ between specialist and generalist bark beetles?*** - Jared Bernard (Virtual)

46. ***Genomic analysis of the UDP-glycosyltransferase gene family in arthropods*** - Seung-Joon Ahn

47. ***A historical review: Non-intact ex vivo tick culture use in tick-borne virus study and functional application*** - Jeffrey M. Grabowski (Virtual)

48. ***Functional characterization of sex-specific yeast interfering RNA larvicides facilitates mosquito sex separation and provides insight into the evolution of dipteran insect sex chromosomes and dosage compensation*** - Teresia Njoroge

49. ***Phenotypic and transcriptomic analyses of abnormal male sexual development in Aedes aegypti and Aedes mascarensis backcross progeny*** - Jiangtao Liang

50. ***Comparisons of human and non-human feeding Anopheles mosquitoes link olfactory genes to anthropophily*** - Luke Ambrose (Virtual)

51. ***CRISPR-Cas9 mutagenesis creates an Aedes aegypti mutant lacking light-evoked behavioral responses*** - Cora E. Anderson

52. ***Genome-wide profiling of Kruppel Homolog 1 (Kr-h1) in Aedes aegypti females throughout egg maturation using CUT&RUN*** - Katara Griffith

53. ***Cytochrome P450s in green peach aphid facilitate its transgenerational tolerance to indole glucosinolate-mediated plant defense*** - Keyan Zhu-Salzman

Microbiome

54. ***Overabundance of Asaia and Serratia bacteria is associated with deltamethrin insecticide susceptibility in Anopheles coluzzii from Agboville, Côte d'Ivoire*** - Bethanie Pelloquin

55. ***Chromosomal-level assembly of Bactericera cockerelli reveals rampant gene family expansions and duplications of horizontally transferred genes that impact insect-microbe-plant-interactions*** - Allison K. Hansen (Virtual)

56. ***Hidden structural diversity within a Wolbachia strain infecting cherry-infesting Rhagoletis (Diptera: Tephritidae) flies across North America*** - Daniel J. Bruzzese

57. ***Delivery of a genetically marked Serratia AS1 to medically important arthropods for use in RNAi and paratransgenic control strategies*** - Mohammad Ali Oshaghi (Virtual)

58. ***Gut microbiota of sand fly vectors of zoonotic visceral Leishmaniasis (ZVL); Host-environment interplay shapes diversity*** - Mohammad Ali Oshaghi (Virtual)

59. ***Microbiome analysis of the New World Screwworm from wild populations, mass-rearing colonies, and transgenic strain*** - Alex P. Arp

60. ***Microbial symbiont curation through innate immune system evolution*** - Kyle Buffin

61. ***Evolution of endosymbiont protein-complexes through label-free proteomics*** - Gerald Maeda

62. ***Symbiont-mediated gene knockdown of honey bees (Apis mellifera)*** - Jo-anne Holley

63. ***An integrated overview of the bacterial flora composition of Hyalomma anatolicum, the main vector of CCHF*** - Mohammad Ali Oshaghi (Virtual)

Platform/Oral Presentations

KEYNOTE LECTURE

Mosquito immune evasion and the dispersal of ancestral *Plasmodium falciparum* and globalization of malaria

Carolina Barillas-Mury and Alvaro Molina-Cruz
Laboratory of Malaria and Vector Research, National Institutes of Health, USA.

Studies with *Plasmodium berghei*, a mouse malaria model, revealed that the mosquito immune system can greatly limit *Plasmodium* infection and that antiplasmodial responses involve the coordinated activation of epithelial, cellular, and humoral immunity. We discovered that *Plasmodium falciparum*, the parasite that causes the most virulent form of malaria, evolved a strategy to evade these responses that is mediated by the *Pfs47* gene. *Pfs47* is a polymorphic surface protein with signatures of diversifying selection and a strong geographic genetic structure at a continental level. We propose that Pfs47-mediated immune evasion has been critical for the globalization of *P. falciparum* malaria, as parasites adapted to new vector species. The "lock and key theory" of *P. falciparum* globalization was proposed. According to this model, one can think of Pfs47 as a "key" that allows the parasite to "turn off" the mosquito detection system by interacting with some mosquito receptor protein(s) (the lock). There are different haplotypes of this "key", and the parasite needs to have the right "key" for the "lock" present in a given mosquito species in order to survive and continue to be transmitted in a new region. The mosquito Pfs47 receptors have been recently identified. *Plasmodium falciparum* malaria originated in Africa, when *Plasmodium praefalciparum*, a gorilla malaria parasite transmitted by sylvan anopheline mosquitoes, adapted to humans. New studies on how the compatibility of ancestral *P. falciparum Pfs47* with the midgut receptors of Asian vectors affected the early dispersal of human malaria to the Asian continent and novel strategies to disrupt malaria transmission that target Pfs47 will be discussed.

PAN-PACIFIC

Genomes of rice insect pests and their parasitic wasps

ABSTRACT TBD

Fei Li
Institute of Insect Science, China

Species divergence in gut-restricted bacteria of social bees

Yiyuan Li[1,2], Sean P. Leonard[1], J. Elijah Powell[1], Nancy A. Moran[1]
[1]Department of Integrative Biology, The University of Texas at Austin, Austin, TX 78712, USA; [2]Current Address: Institute of Plant Virology, Ningbo University, Ningbo, China

Host-associated microbiomes, particularly gut microbiomes, often harbor related but distinct microbial lineages, but how this diversity arises and is maintained is not well understood. A prerequisite for lineage diversification is reproductive isolation imposed by barriers to gene flow. In host-associated microbes, genetic recombination can be disrupted by confinement to different hosts, for example following host speciation, or by niche partitioning within the same host. Taking advantage of the simple gut microbiome of social bees, we explore the diversification of two groups of gut-associated bacteria, *Gilliamella* and *Snodgrassella*, which have evolved for 80 million years with honey bees and bumble bees. Our analyses of sequenced genomes show that these lineages have diversified into discrete populations with limited gene flow. Divergence has occurred between symbionts of different host species and, in some cases, between symbiont lineages within a single host individual. Populations have acquired genes to adapt to specific hosts and ecological niches; for example, *Gilliamella* lineages differ markedly in abilities to degrade dietary polysaccharides and to use the resulting sugar components. Using engineered fluorescent bacteria in vivo, we show that *Gilliamella* lineages localize to different hindgut regions, corresponding to differences in their abilities to use spatially concentrated nitrogenous wastes of hosts. Our findings show that bee gut bacteria can diversify due to isolation in different host species and also due to spatial niche partitioning within individual hosts, leading to barriers to gene flow.

Genomic hijacking - how parasitic worms manipulate their insect hosts

Eddy Dowle
Department of Anatomy, University of Otago, Dunedin

Parasites routinely manipulate the behaviour of their hosts to enhance their survival and transmission. One of the most extraordinary of these host manipulations is the water-seeking behaviour that some nematodes and hairworms induce in their insect hosts so that the worms might exit the host in a suitable environment and reproduce. The worm hijacks the host's central nervous system forcing the normally terrestrial host to seek water. Once water is found the adult worm, often equal to or bigger than the host, erupts in an explosive frenzy, sacrificing the host, so that the parasite might complete its lifecycle. This amazing alteration in behaviour is induced by worms spanning two phyla (Nematoda and Nematomorpha) and is observed in a variety of arthropod hosts, notably crickets, wētā and earwigs. Host manipulations are the consequence of genes in the parasite genome modifying the hosts' phenotypic traits. But the development and genetic control of these behavioural modifications are not well understood as experimentally tractable systems are rare. Here we are combining genome assembly with whole transcriptome analysis to assess transcriptomic changes in the host brain as the parasites develop immature (non-manipulative) to mature (manipulative) worms. We show how the complex relationship between parasite and host develops from early to late infection and begin to elucidate the genetic mechanisms by which the hosts fatal water-seeking behaviour is induced.

What forces shape you? The genomics to the Dark Side

Lifei Qiu, Shuai Wang, Shanlin Liu and **Xin Zhou**
Department of Entomology, China Agricultural University

The Asian honeybee, *Apis cerana*, is one of the only two extant honeybee species that have successfully expanded their range from Asian tropical to the temperate. Its widespread range has largely overlapped with Asia's major agricultural region, therefore making significant contribution in pollination. Our recent study on the mainland *A. cerana* indicated that the current population structure and distribution pattern is a result of repeated range expansion and retraction, in response to climatic oscillation of the ice age. In particular, the common ancestral population has independently invaded different valleys of the eastern and southern Himalayans, forming local populations adaptive to mountain habitats. A shared morphological change among mountain honeybees is the convergent elevation in body pigmentation. Although generally assumed as an adaptation to low temperature, it remains unclear how does the darkened coloration improve the fitness of individual foragers and the entire colony, and what the underlying molecular mechanism is. Here we employed 3 pairs of mountain-plain *A. cerana* populations and ask: 1) how does darkened body coloration benefit the species at low temperature? 2) whether the same genes are responsible for the convergent morphological change and how could natural selection repeatedly work on the same trait/gene? We showed that the darkened foragers increased body temperature more rapidly under rising ambient temperature, and eventually reached a higher balanced temperature. In addition, common garden experiments demonstrated that heat lost during foraging was significantly decreased in darkened foragers, which would in-turn improve thermal regulation of the colony. Comparative genomics analyses suggested that the *mycC* gene, an ortholog of ebony, was repeatedly selected among the 3 independent mountain populations. The expression pattern of *mycC* was in concordance with the pigmentation process during pupual development. And *mycC* RNAi led to apparent increase in body darkness, confirming its role in shaping body coloration. Further fine screening of the *mycC* sequence revealed that selection signals were condensed in its upstream regulation region, with varying sites among populations. These results indicated that the mountain honeybees had evolved improved capability in maintaining colony temperature via darkened body coloration of individual foragers. This parallel adaptive trait was enabled by natural selection independently operating on the upstream of the pigmentation gene, *mycC*, presumably through adjustments of expression levels.

I5k/EMERGING GENOMES

The assembly of the rice weevil *Sitophilus oryzae* is dominated by transposable elements and shades light on the evolution of endosymbiosis in a major crop pest.

Nicolas Parisot*, Carlos Vargas-Chávez*, **Clément Goubert*,** Patrice Baa-Puyoulet, Séverine Balmand, Louis Beranger, Caroline Blanc, Aymeric Bonnamour, Matthieu Boulesteix, Nelly Burlet, Federica Calevro, Patrick Callaerts, Théo Chancy, Hubert Charles, Stefano Colella, André Da Silva Barbosa, Elisa Dell'Aglio, Alex Di Genova, Gérard Febvay, Toni Gabaldón, Mariana Galvão Ferrarini, Alexandra Gerber, Benjamin Gillet, Robert Hubley, Sandrine Hughes, Emmanuelle Jacquin-Joly, Justin Maire, Marina Marcet-Houben, Florent Masson, Camille Meslin, Nicolas Montagné, Andrés Moya, Ana Tereza Ribeiro de Vasconcelos, Gautier Richard, Jeb Rosen, Marie-France Sagot, Arian F. A. Smit, Jessica M. Storer, Carole Vincent-

Monegat, Agnès Vallier, Aurélien Vigneron, Anna Zaidman-Rémy, Waël Zamoum, Cristina Vieira**, Rita Rebollo**, Amparo Latorre** & Abdelaziz Heddi**
*Co-first authors; **Co-corresponding authors

The rice weevil *Sitophilus oryzae* is a major agricultural pest, causing extensive damage to cereal, both in the field and storages. The adaptive success of *S. oryzae* is fostered by an intracellular symbiotic relationship (endosymbiosis) with the Gram-negative bacterium *Sodalis pierantonius* which provides vitamins and essential amino acids to the insect. Thus, *S. oryzae* represents a unique model to decipher the evolution of host-symbiont molecular interactions. Here, we present the first genome assembly of *S. oryzae*, built upon a combination of short and long reads. Our assembly spans 770 Mbp, which matches estimates obtained by flow cytometry. We discovered that *S. oryzae* has accumulated an astonishing amount of transposable elements (TE), adding up to 72% of the genome. Given the relevance of TEs in the evolution of eukaryotic genomes, we tackled the challenging task of annotating thousands of diverse TE families by developing an *ad-hoc* strategy involving bioinformatic automatisation and manual curation. The genes' architecture of *S. oryzae* presents striking similarities with TE-rich mammalian genomes, but the average age of these repeated elements suggest a much higher turnover of these sequences, as observed in other insect models. Many TE families are transcriptionally active, several of them varying in expression between guts and ovaries, and the presence or absence of the insect's endosymbiont. Furthermore, we show that *S. oryzae*'s genome includes complete small RNA pathways with genes mainly expressed in the gonads relative to the soma, suggesting an active repression of TEs in these tissues. Using low-coverage sequencing of related *Sitophilus* species, we observed a conservation of the TE landscape with the notable exception of *S. linearis*, the only non-granivorous species considered. Taken together, these discoveries highlight the central place of TEs in the evolution of *S. oryzae*'s genome, and suggest that close interactions between host genome, TE content and the endosymbiont may be a favorable strategy to the ecological success of the holobiont. This work establishes a reference genome for an agricultural pest beetle, which may act as a foundation for pest control and the study of endosymbiosis and TE evolution and regulation.

The Pest Genomics Initiative

Robert King, David Hughes, Emma Bailey, Benjamin Buer, Chris Rawlings, Crossthwaite Andrew GBJH, Emyr Davies, Ganko Eric USRE, Guest Marcus GBJH, Isolde Haeuser-Hahn, Keywan Hassani-Pak, Klaus Raming, Martin Williamson, Ralf Nauen, Saskia Ruehmer, Zimmer Christoph CHST, Lin Field

As the world population grows – along with demands for high-quality, nutritious food, produced with less impact on the environment - there is a need for new pest-control strategies that can both reduce crop losses and conserve biodiversity and ecosystems. These goals can benefit greatly from the availability of high-quality genomic resources. With this in mind, research leaders from Rothamsted Research Ltd., Bayer Crop Science and Syngenta Crop Protection worked together on a Pest Genomics Initiative (PGI) which sequenced and annotated the genomes of 20 key global pests and 3 beneficial insects. The results from this pre-competitive early stage research collaboration will be released into the public domain on 14th June 2022 for the benefit of all. There are many opportunities for using high quality insect genomic data to aid the design of better, more selective crop protection compounds, for better mitigation of the effects of resistance, and for the development of biologicals and improved traits for insect control. Such genomic resources will also open up exciting new areas that are not being widely exploited at present. What is needed is high quality genome data from insect strains that are well characterized, and that are of high quality in terms of both the assembled genome and the annotated gene models, to have both high

representation and high accuracy genes. As well as industrial research labs needing high quality information for developing novel pest control technologies, these resources will benefit academic research in many areas of entomology. Data can be found at https://www.pestgenomics.org/.

i5k in the landscape of global, regional, and national biogenome initiatives

Robert M. Waterhouse
Department of Ecology and Evolution, University of Lausanne, and the Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland

Arthropod genomes are critical for performing cutting-edge entomological research, from gaining key insights into basic animal biology, to finding new ways to limit the effects of damaging species or protect threatened ones, and to understand the evolution of arthropod diversity on Earth. The i5k initiative to coordinate the sequencing, assembly, annotation, and analysis of 5,000 arthropod genomes was officially launched just over a decade ago (Robinson et al. 2011). The i5k pilot project formed a natural focal point for community engagement, culminating with the production of genomic resources for 28 species presented collectively in the analyses of Gene Content Evolution in the Arthropods (Thomas *et al.* 2020). In parallel, other BioGenome projects of varied taxonomic or geographic scope have been launched that have clear synergies with the overarching goals of the i5k initiative. The umbrella Earth BioGenome project (EBP, www.earthbiogenome.org) aims to coordinate the sequencing and characterisation of the genomes of all of Earth's eukaryotic biodiversity. With a network of networks model the EBP connects BioGenome initiatives such as the Africa BioGenome Project (https://africanbiogenome.org), the Ag100 Pest Initiative of the USDA (http://i5k.github.io/ag100pest), the Darwin Tree of Life project (DToL, www.darwintreeoflife.org), and the European Reference Genome Atlas (ERGA, www.erga-biodiversity.eu) initiative. This landscape of BioGenome Initiatives can seem difficult to navigate, particularly for newcomers to the i5k Arthropod Genomics Community. Here we aim to provide an introductory map that outlines ongoing efforts most relevant to the Arthropod Genomics Community as well as highlighting the tools and resources being developed to overcome scientific and organisational challenges.

Targeted sequencing detects candidate resistance alleles to *Bacillus thuringiensis* insecticidal proteins in invasive *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Peter Tandy[1], Kurt Lamour[1], Rodney Nagoshi[2], Caroline Placidi de Bortoli[1], **Scott Emrich[3]**, and Juan Luis Jurat-Fuentes[1]
[1]Department of Entomology and Plant Pathology, University of Tennessee, USA; [2]Center for Medical, Agricultural and Veterinary Entomology, United States Department of Agriculture-Agricultural Research Service, USA; [3]Department of Electrical Engineering and Computer Science, University of Tennessee, USA

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a highly polyphagous pest native to the tropical Americas that in the last 5 years has spread to become a global superpest threatening food and fiber production. Transgenic crops producing insecticidal Cry and Vip3Aa proteins from the bacterium *Bacillus thuringiensis* (Bt) have been used for effective control of this pest in North and South America. Evolution of practical resistance to these Bt crops represents the greatest threat to sustainability of this technology and its potential use in controlling *S. frugiperda*. Monitoring for resistance is vital to management approaches

intended to delay resistance to Bt crops in *S. frugiperda*. Current monitoring efforts focus on bioassays with *S. frugiperda* larvae derived from field-collected parents. DNA-based methods present higher sensitivity and cost-effectiveness compared to current bioassay-based resistance monitoring. Targeted sequencing of the SfABCC2 gene linked to practical resistance against Cry1F corn in Puerto Rico confirmed high frequency of a known resistance allele in this population and provided novel candidate resistance alleles. In this study, we performed targeted SfABCC2 sequencing to detect known and candidate resistance alleles in field-collected *S. frugiperda* from continental USA, Africa (Ghana, Togo and South Africa) and Southeast Asia (Myanmar). Results identify additional candidate resistance alleles in the invasive *S. frugiperda* range but did not detect the previously known allele outside the Caribbean. Conservation of some of these alleles among samples from diverse locations contributes to our understanding of *S. frugiperda* spread.

STRUCTURAL EVOLUTION

Using bioinformatics and RNA interference to investigate the genetic architecture of body plan patterning in spiders

Emily Setton
University of Wisconsin-Madison

The phylum Arthropoda is notable for myriad structural innovations and body plan disparity, making it a choice system for studying the developmental genetic basis underlying novel structures and body plan patterning. A subset of chelicerate arthropods that includes spiders and scorpions is especially attractive for understanding how new genes acquire or subdivide ancestral functions, because this group of arachnids is known to have undergone an ancient shared whole genome duplication event. To understand the spatiotemporal dynamics of new gene copies in spiders, we created a high-quality developmental transcriptome for the tarantula *Aphonopelma hentzi* and applied a differential gene expression approach (DGE) to specific regions of embryonic tissue at selected stages. We thereby generated a comprehensive library to investigate candidate genes involved in patterning axes, as well as novel appendage types restricted to arachnids (e.g., chelicerae; palps; book lungs; spinnerets). We tested the predictions of the DGE approach using parental RNAi in the spider model *Parasteatoda tepidariorum*. Here, we show that one spider copy of the Iroquois-class transcription factor (*Irx4*) is required for patterning the dorso-ventral axis of the segments spanning the boundary of the two tagmata of spiders. Intriguingly, the segments affected by RNAi are positionally homologous to the thoracic segments of the fruit fly *Drosophila melanogaster*, suggesting deeply conserved dynamics of axis patterning in these two groups, despite the differences in tagmatic boundaries.

The evolution of interspecific reproductive incompatibilities in *Drosophila*

Yasir Ahmed-Braimah
Department of Biology, Syracuse University

One of the fundamental problems in evolutionary biology is to understand the molecular genetic basis of speciation. In the handful of cases where the genetic mechanisms of reproductive isolation have been elucidated, these invariably tackle post-zygotic reproductive barriers. This means that we still lack a general understanding of the molecular processes that govern pre-zygotic reproductive barriers, even though these

are often important early in the speciation process. My lab seeks to identify the molecular genetic basis of pre-zygotic reproductive isolation between members of the *Drosophila virilis* species sub-group. This species group provides an especially unique opportunity to dissect the genetic and molecular mechanisms of pre-zygotic barriers, as members of this group are prone to evolve these types of barriers quickly between species and even among populations of the same species. Our overall approach integrates several strategies to answer the following questions: What are the genetic mechanisms that cause reproductive isolation between species? Which molecular and cellular processes are affected by divergence of these genetic mechanisms? What are the evolutionary forces that drive divergence of the relevant genes between species? What is the landscape of natural genetic variation within and between species that facilitates evolutionary divergence of these genes?

Discovery of large chromosomal inversions in the genome of *Aedes aegypti* across the tropics

Jiangtao Liang[1], Andrey Yurchenko[1], Varvara Lukyanchikova[1], Ilya Brusentsov[2], Noah Rose[3], Zhijian Tu[4], Carolyn McBride[3], and Maria Sharakhova[1,2]
[1]Department of Entomology and Fralin Life Science Institute, Virginia Polytechnic and State University, Blacksburg, USA; [2]Laboratory of Cell Differentiation, the Federal Research Center, Institute of Cytology and Genetics, Novosibirsk, Russia; [3]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, USA. [4]Department of Biochemistry and Fralin Life Science Institute, Virginia Polytechnic and State University, Blacksburg, USA

Polymorphic chromosomal inversions have been shown to be associated with adaptations and traits relevant to malaria transmission in anopheline mosquitoes. However, the discovery of chromosomal inversions in aedini mosquitoes has been challenging because of the absence of high-quality polytene chromosomes. In this study, we applied a Hi-C approach to 22 strains of arbovirus vector *Aedes aegypti* from 13 countries across the tropics. Totally, 20 multi-megabase chromosomal inversions with sizes varying from 1.5 to 55 Mb were found. The presence of inversions was validated by fluorescence in situ hybridization in mitotic chromosomes. Interestingly, inversions found in *Ae. aegypti* were not distributed along chromosomes evenly and were more abundant in 1q and 3p arms, which are homologous to the inversion-rich 2R arm in the malaria vector *Anopheles gambiae*. Inversions were more abundant in populations from Western Africa where the highest number of them was observed. Our results suggest an existence of a large pool of structural variations in the *Ae. aegypti* genome potentially involved in the adaptation and pathogenesis of this mosquito.

Paternal genome elimination creates contrasting evolutionary patterns in male and female citrus mealybugs

Andrew J. Mongue and Laura Ross
Institute of Evolutionary Biology, University of Edinburgh, UK

Mealybugs are a uniquely interesting group of insects, both as crop pests and as exhibitors of a strange form of reproduction known as Paternal Genome Elimination (PGE). Males of this system are functionally haploid, with their father's genetic contribution transcriptionally repressed, and can only pass on their maternally inherited genes to offspring. This unusual method of reproduction should create drastically different selective pressures for males and females, but has never been studied in a population genomic framework. For the first time, we have generated whole genome resequencing data for multiple wild

populations of *Planococcus citri*, the citrus mealybug as well as sex- and stage-specific RNA sequencing. We show that adult males and females have incredible sexual dimorphism in gene expression and rates of adaptive evolution strongly differ for genes expressed in male- and female-specific patterns. Furthermore, comparisons between populations offer insights on the spread of these globally distributed pest insects, making these data and results valuable in both basic and applied contexts.

VECTOR GENOMICS

Genomic basis of transitions between human-specialist and generalist ecology in *Ae. aegypti*'s native range

Noah Rose
Department of Ecology and Evolutionary Biology, Princeton University

Of thousands of species of mosquitoes, just a handful present the greatest threats to public health: those that have recently evolved a strong preference for human hosts and habitats. We recently examined the ecological drivers such a shift in the dengue, Zika, and yellow fever vector *Aedes aegypti*, finding that both dry season intensity and rapid urbanization have likely contributed to specialization on humans within Africa. Here I will discuss the genomic origins of these shifts in ecology. A large number of genes are involved in specialization on humans, but they are concentrated in just a few key genomic loci. A large chromosomal inversion spans the most important locus, and may facilitate rapid shifts in ecology at the sharp geographic transition between differently adapted populations in Senegal.

A transcriptomic approach to mosquito anatomy, physiology and immunity

B Hixon and **Nic Buchon**

Cornell Institute of Host Microbe Interactions and Disease, Dept of Entomology, Cornell University, Ithaca, NY. Email: nicolas.buchon@cornell.edu

Mosquitoes transmit numerous pathogens, but large gaps remain in our understanding of their physiology. To facilitate explorations of mosquito biology, we have created Aegypti-Atlas (http://aegyptiatlas.buchonlab.com/), an online resource hosting RNAseq profiles of *Ae. aegypti* body parts (head, thorax, abdomen, gut, Malpighian tubules, ovaries), gut regions (crop, proventriculus, anterior and posterior midgut, hindgut), and a gut time course of blood meal digestion. Using Aegypti-Atlas, we provide insights into regionalization of gut function, blood feeding response, and immune defenses. We find that the anterior and posterior midgut possess digestive specializations which are preserved in the blood-fed state. Blood feeding initiates the sequential induction and repression/depletion of multiple cohorts of peptidases. With respect to defense, immune signaling components, but not recognition or effector molecules, show enrichment in ovaries. Basal expression of antimicrobial peptides is dominated by holotricin and gambicin, which are expressed in carcass and digestive tissues, respectively, in a mutually exclusive manner. In the midgut, gambicin and other effectors are almost exclusively expressed in the anterior regions, while the posterior midgut exhibits hallmarks of immune tolerance. Finally, in a cross-species comparison between Ae. aegypti and Anopheles gambiae midguts, we observe that regional digestive and immune specializations are conserved, indicating that our dataset may be broadly relevant to multiple mosquito species. We demonstrate that the expression of orthologous genes is highly correlated, with the exception of a 'species

signature' comprising a few highly/disparately expressed genes. With this work, we show the potential of Aegypti-Atlas to unlock a more complete understanding of mosquito biology.

Thrips (Thysanoptera: Paraneoptera) have an ancient relationship with their vectored tospoviruses: a genomic approach to predicting hidden vectors of disease

David A. C. Stanford-Beale and Stephen L. Cameron
Department of Entomology, Purdue University, West Lafayette, Indiana, 47907 USA

Thrips, Thysanoptera (Paraneoptera), are an enigmatic order of minute insects that vector the damaging group of crop plant viruses, tospoviruses (Tospoviridae). Investigating the origins of tospovirus vectoring in thrips has previously been hampered by the lack of support for early relationships in both the thrips and tospovirus phylogenies. A candidate gene for endoCP-GN, a possible tospovirus binding cuticle protein, has been described in the *Frankliniella occidentalis* genome. Whether or not this gene is found in other taxa was previously unknown. Using a cophylogenetic and genomic approach, we provide evidence for an ancient relationship between thrips and tospoviruses (~121 m.y.a.). We examine the cophylogeny using four different evolutionary scenarios, approximate to hypotheses present in the literature, and provide comment on the evolutionary history between thrips and their tospoviruses. We confirm the presence of assembled endoCP-GN homologs across representatives of Thripidae. We predict that tospovirus vectoring, and the potential to vector can be inferred from assembled sequences and call for the field to concentrate efforts on non-pest species of thrips that may be reservoir vectors of tospoviruses.

Evolutionary characterization of innate immune related genes in six *Glossina* species

Pauline Karega[1], Robert Waterhouse[2], Rosaline Macharia[1] and Dan Masiga[3]
[1]University of Nairobi; [2]University of Lausanne; [3]International Centre for Insect Physiology and Ecology

Tsetse flies are vectors of trypanosomes that cause Animal and Human African trypanosomiasis. Trypanosomiasis has been implicated in losses of up to 4.75 billion US dollars in the agricultural and economic sectors. Tsetse immunity plays a role in the vector competence of *Glossina* species, with presumably different environmental and microbial exposures leading to evolutionary changes in their repertoires of immune genes. Advancement in sequencing technologies has made possible the sequencing of whole genomes of various insect species including tsetse flies, facilitating comparative genomics studies of dipteran immune-related genes. Such initial analyses have revealed gene family expansions, contractions and gene losses in *Glossina* relative to *Musca domestica* and *Drosophila melanogaster*. However, current annotated gene models from six *Glossina* genomes require careful curation and systematic annotation for comprehensive downstream analysis. This project therefore proposed to evaluate a bioinformatics workflow that automates the process of immune gene identification and signature characterization, following manual annotation. The objectives of this study included to characterize evolutionary features of immune related genes in tsetse flies, determine the timings of gains and losses of immune related genes on the *Glossina* phylogeny and to evaluate a workflow for automated identification and characterization. Using the *Glossina morsitans morsitans* genome as a reference, manual curation of selected immune-related genes was first performed. This allowed for accurate gene prediction in the other species based on the high-quality curated gene models. Subsequent phylogenetic analyses of these genes were then performed to infer orthology and

paralogy, from which gene gain and loss events were identified, and sequence evolutionary rates and constraints estimated. A relaxed molecular clock species phylogeny was then used to date the gene gain and loss events. These evolutionary features helped to understand the evolution of *Glossina* immunity and also shed light on the evolutionary relationships with other dipterans such as *Drosophila*, *Musca* and *Anopheles*. Automation of this process will also allow faster characterization of such evolutionary features of other gene families and other insects in the future. This study explored evolutionary features of immune-related genes in *Glossina*, provided accurate timings of changes in the immune repertoire across six *Glossina* species, and will develop a tool to improve future characterizations of evolutionary features in newly sequenced genomes.

AFRICAN GENOMICS

The PAMCA vector genomics surveillance program: an overview

Elijah Juma
Pan African Mosquito Control Association, Africa

ABSTRACT TBD

Malaria vector surveillance and population genetics characterization in Central Africa

Sandrine Nsango
University of Douala, Cameroon

ABSTRACT TBD

Updates on ongoing evaluation of vector control interventions and operational research around vector surveillance

Eric Ochomo
Kenya Medical Research Institute, Kenya

ABSTRACT TBD

Population genetic structure and diversity for *Anopheles funestus* based on mitochondrial DNA markers (mtDNA-COI and mtDNA-COII) in western Kenya

Isaiah Debrah[1,2], Kevin O. Ochwedo[2], Maxwell G. Machani[3], Shirley A. Onyango[2], Daibin Zhong[4], Linda E. Amoah[5], Andrew K. Githeko[3], **Yaw A. Afrane[6]**, and Guiyun Yan[4]
[1]West Africa Centre for Cell Biology of Infectious Pathogen, University of Ghana, Accra, Ghan;, [2]Sub-Saharan Africa International Centre of Excellence for Malaria Research, Homabay, Keny;, [3]Kenya Medical Research Institute, Kisumu, Kenya; [4]Program in Public Health, College of Health Sciences, University of

California at Irvine, Irvine, CA, United States of America; [5]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana; [6]Department of Medical Microbiology, University of Ghana Medical School, College of Health Sciences, University of Ghana, Accra, Ghana

In this study, the mitochondrial markers, cytochrome oxidase subunits I (mtDNA-COI) and II genes (mtDNA-COII) were used to assess the genetic structure and diversity of *Anopheles funestus*, a very important malaria vector in Africa, that adapt and colonize different ecological niches in western Kenya. Adult mosquitoes were collected using mechanical aspirators in four sites (Bungoma, Port Victoria, Kombewa and Migori) across four counties in western Kenya. All samples were morphologically identified as *An. funestus* s.l. The mtDNA-COI and mtDNA-COII genes were amplified, sequenced and analyzed to identify sibling species and the genetic structure of the *Anopheles funestus* population. Hundred and Sixty (160) *An. funestus* s.l. specimens (40 from each site) were used for species identification, genetic structure and gene flow. The mtDNA-COI gene showed that *An. funestus* constitutes 96% of all the specimens identified. Other members of the *An. funestus* complex identified includes *Anopheles funestus*-like *Anopheles longipalpis*, *Anopheles parensis* and *Anopheles vaneedeni*. Both genes exhibit high haplotype diversity (COI, Hd=0.99; COII, Hd=0.98) but low nucleotide diversity (mtDNA-COI, $\hat{I}$ =0.03; mtDNA-COII, $\hat{I}$ =0.02). A minimal level of genetic differentiation was observed between Port Victoria and Bungoma (Fst = 0.01512, P= 0.00000), Port Victoria and Kombewa (Fst = 0.03135, P=0.00000), Migori and Kombewa (FST = 0.03568, P=0.000), Migori and Bungoma (Fst = 0.01621, P=0.01802) but no genetic differentiation between Port Victoria and Migori (FST = 0.00319, P= 0.180) and Kombewa and Bungoma (FST = 0.01121, P=0.0900).The high gene flow (Nm) was between Port Victoria and Migori (Nm=81.79) and low gene flow was observed between Migori and Kombewa (Nm=6.17). Neutrality tests suggest population expansion of *Anopheles funestus* with an excess of low-frequency variation. This is the first report on *Anopheles funestus*-like in western Kenya. No barrier to gene flow was observed in *Anopheles funestus* population. Population expansion suggests the high adaptability of this species.

ARTHROPOD OMICS

Viral infection impacts transposable element transcript amounts in *Drosophila*

Marie Fablet
Biometry & Evolutionary Biology Department, Claude Bernard University

Transposable elements (TEs) are genomic parasites that are found in all genomes, some of which display sequence similarity to certain viruses. In insects, TEs are controlled by the Piwi-interacting small interfering RNA (piRNA) pathway in gonads, while the small interfering RNA (siRNA) pathway is dedicated to TE somatic control and defense against viruses. So far, these two small interfering RNA pathways are considered to involve distinct molecular effectors and are described as independent. Using Sindbis virus (SINV) in Drosophila and high throughput RNA sequencing, here we show that viral infections affect TE transcript amounts via modulations of the piRNA and siRNA repertoires, with the clearest effects in somatic tissues. These results suggest that viral acute or chronic infections may impact TE activity and, thus, the tempo of genetic diversification.

Analysis of the *Aedes aegypti* gonotrophic cycle using synchrotron x-ray microCT

Lindsey Mack
University of California-Davis

Traditional methods of viewing the internal anatomy of insects require some degree of tissue manipulation and/or destruction. Through the use of synchrotron based x-ray phase contrast microCT (pcMicroCT), avoids this issue and has the capability to produce high contrast, three dimensional images . Our lab is using this technique to study the morphological changes occuring in the mosquito *Aedes aegypti* during its reproductive cycle. *Ae. aegypti* is the primary global arbovirus vector, present on all continents except Antarctica. Their ability to spread these viruses is tightly linked with their ability to reproduce, as the production of eggs in this species is initiated by blood feeding. Amazingly, this species produces a full cohort of eggs (typically 50-100) in just 3 days time following a blood meal. This rapid development represents dramatic shifts in physiological processes that result in massive volumetric changes to internal anatomy over time. To explore these changes thoroughly, a time course of microCT scans were completed over the vitellogenic period. This dataset provides a virtual representation of the volumetric, conformational, and positional changes occuring in the gut and ovaries of mosquitoes across the vitellogenic period. This dataset provides the field of vector biology with a detailed three dimensional internal atlas of the processes of vitellogenesis in *Ae. aegypti*. Thus far, segmentation of the gut and ovaries has been completed, with plans to perform detailed segmentation of the fat body.

Assembly and characterization of W chromosome in monarch butterfly (*Danaus plexippus*)

Martina Dalikova[1], Daniel Portik[2], Sarah B. Kingan[2], Hayley Mangelson[3], Liuqi Gu[1], James R. Walters[1]
[1]Department of Ecology and Evolutionary Biology, The University of Kansas, Lawrence, KS 66045, USA; [2]Pacific Biosciences, Menlo Park, CA 94025, USA 3 Phase Genomics, Seattle, WA 98109, USA

Degenerated sex chromosomes have a precedent of being notoriously difficult to assemble. Recent advances in long-read sequencing are catalyzing increases in the number and quality of W and Y assemblies, providing novel insights concerning the content and evolution of these elusive chromosomes. Here we report a novel genome assembly for the monarch butterfly (*Danaus plexippus*), focusing on the W chromosome. This species harbors a neo-Z chromosome, arising from the fusion of the ancestral Z with an autosome. Previous cytogenetic analyses indicated a similarly large and bipartite W chromosome, suggesting the possibility of a comparable neo-W, but much ambiguity remains concerning the sequence and history of monarch W chromosome. We generated PacBio HiFi reads with Hi-C data from females to support *de novo* assemblies of maternal and paternal haplotypes using Trio binning. Putative W contigs from the maternal genome were determined based on male to female coverage and sex specific kmers. The paternal assembly and W contigs were scaffolded using Hi-C data. This produced chromosomal level scaffolds for the Z and all autosomes, including three autosomes assembled from telomere to telomere. The W chromosome scaffold length was around 10Mbp, thus leaving about one third of this chromosome in unscaffolded contigs. While primarily composed of transposable elements (TEs), the W contains at least two protein coding genes that arose through retroposition and ectopic recombination from other chromosomes. Neither of these W-linked copies appear pseudogenized and one appears to be evolving adaptively. The prevalent repetitive content of the W chromosome are elements from the LINE and LTR

retrotransposon groups. Surprisingly, the TEs on W chromosome have lower divergence compared to the rest of the genome, suggesting their ongoing accumulation. Finally, despite this novel W assembly, strong evidence for or against a neo-W chromosome origin remains elusive.

Schistocerca (Orthoptera: Acrididae) as a model clade for studying phenotypic plasticity from a genomic perspective

Hojun Song[1,5], Anna Childers[2,5], Olga Dudchenko[3,5], Scott Geib[2], Sheina Sim[2], Tyler Simmonds[2], Amanda Stahlke[2,5], Maeva Techer[1,5], Stephen Richards[3,5], Erez Lieberman Aiden[3,5], and Fabrizio Gabbiani[4,5]
[1]Department of Entomology, Texas A&M University, College Station, TX, USA; [2]United States Department of Agriculture, Agricultural Research Servic;e [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [4]Department of Neuroscience, Baylor College of Medicine, Houston, TX, USA; [5]NSF-BII Behavioral Plasticity Research Institute

The genus *Schistocerca* (Orthoptera: Acrididae) includes some of the most devastating locust species in the world, including the desert locust (*S. gregaria*), the Central American locust (*S. piceifrons*), and the South American locust (*S. cancellata*). These locust species show an extreme form of density-dependent phenotypic plasticity in which cryptic and shy individuals, known as the solitarious phase, can transform into conspicuous and gregarious individuals, known as the gregarious phase, in response to changes in local population density. In fact, this "locust phase polyphenism" is what makes the locusts distinctly different from regular grasshoppers. Intriguingly, *Schistocerca* includes 45 species, most of which are non-swarming sedentary grasshopper species, and phylogenetic studies have shown that the locust species do not form a monophyletic group, suggesting that locust phase polyphenism has evolved multiple times in the genus. Furthermore, recent experimental studies have indicated that some of the non-swarming grasshopper species show reduced density-dependent phenotypic plasticity, suggesting that *Schistocerca* as a whole is an exciting model clade that can be used to study how phenotypic plasticity has evolved as species diverge. Until now, it has been very challenging to study these insects from a genomic perspective because of their large genome sizes (up to 9.1 Gb), which are the largest among insects. Recently, however, we launched the Behavioral Plasticity Research Institute (BPRI) supported by the National Science Foundation Biology Integration Institute (NSF-BII) program, which allowed us to produce high-quality chromosome-length assemblies of six Schistocerca species, including three locust and three non-swarming grasshopper species. These genomes were generated using PacBio HiFi and Hi-C technologies with a target coverage of 30x and 15x, respectively. Our assemblies are highly contiguous (contig N50 ranging from 27.4 to 74.2 Mb and scaffold N50 ranging from 791.2 to 854.0 Mb) and consistent with known estimates of chromosome count (11 autosomal pairs + sex chromosome) for these species. In this presentation, we introduce these new genomes as well as other omics approaches that we are currently pursuing, including transcriptomics, epigenomics, and single-cell genomics. We also introduce the overall vision and research activities of the BPRI, and make a strong case for why *Schistocerca* is an excellent model clade for biological integration.

FUNCTIONAL GENOMICS

Stress responses elicit a noncanonical mode of IMD pathway activation in ticks

Lindsay C Sidak-Loftis[1], Kristin L Rosche[1], Natasha Pence[2#], Jessica K Ujczo[3], Joanna Hurtado[1,4], Elis Fisk[1], Alan G Goodman[4], Susan M Noh[1,3], John W Peters[2], **Dana K Shaw[1,4]**
[1]Veterinary Microbiology and Pathology, Washington State University, Pullman, WA; [2]Institute of Biological Chemistry, Washington State University, Pullman, WA; [3]United States, Department of Agriculture, Agricultural Research Service, Animal Disease Research Unit, Pullman, WA; [4]School of Molecular Biosciences, Washington State University, Pullman, Washington, USA. [#]Present address: Department of Chemistry, Dartmouth College, Hanover, NH, USA.

The North American deer tick, *Ixodes scapularis*, transmits 7 pathogens relevant to human health including *Borrelia burgdorferi* (Lyme disease) and *Anaplasma phagocytophilum* (Anaplasmosis). The ability of an arthropod to harbor and transmit pathogens is termed "vector competency". The arthropod immune system can influence vector competency, but the molecular details of tick immunity remain vague. For example, the immune deficiency (IMD) pathway is a defense mechanism that has been well-characterized in insects and senses/responds to Gram negative bacteria. However, ticks lack genes encoding upstream components that initiate the IMD pathway. Despite this deficiency, core IMD pathway signaling molecules are present and functionally restrict Gram negative-like bacterial pathogens *B. burgdorferi* and *A. phagocytophilum*. The molecular events leading to IMD pathway activation in ticks has remained unclear. We have found that a specialized cellular stress-response system termed the Unfolded Protein Response (UPR) functions upstream to activate the IMD pathway. The endoplasmic reticulum (ER) stress receptor, IRE1α, is phosphorylated in response to tick-borne bacteria, but does not splice the mRNA encoding XBP1. Instead, through protein modeling and reciprocal pulldowns, we show that *Ixodes* IRE1α complexes with TRAF2. Disrupting IRE1α-TRAF2 signaling blocks IMD pathway activation and diminishes the production of antimicrobial effectors. Through *in vitro*, *in vivo* and *ex vivo* techniques we demonstrate that the UPR-IMD pathway circuitry limits *B. burgdorferi* and the rickettsial agents *A. phagocytophilum* and *A. marginale*. Collectively, our findings provide an explanation for how the core IMD pathway is activated independent of canonical upstream regulators.

From genome to phenome: tools to understand the basic biology of ticks

Arvind Sharma, Michael Pham, Won-Choel Yim, Robert Harrell, Jason Rasgon, Andrew B. Nuss, **Monika Gulia-Nuss***
Department of Biochemistry and Molecular Biology, University of Nevada-Reno; *Presenting author; Email: mgulianuss@unr.edu

Ticks are obligate hematophagous parasites and are important vectors of a wide variety of pathogens. Lyme disease (LD) caused by the spirochete *Borrelia burgdorferi* and vectored by black-legged tick, *Ixodes scapularis*, is the most prevalent vector-borne disease in the United States. Despite their importance, our knowledge of the biology of the ticks on a molecular level is limited. Advances in tick genomics and genetics have mainly been stymied by 1) lack of molecular tools to carry out forward and reverse genetics and 2) fragmented genome assembly. Tick genome sequencing has been challenging due to a large (>2 gbp) genome size with long repetitive sequences. The currently available *I. scapularis* genome comprises of 369,495 scaffolds representing 57% of the genome. The fragmented genome further poses challenges in identifying gene sequences and therefore a high-quality genome sequence is needed for advance genomics and genetics work in this vector.

To fill these gaps, we have developed methods for targeted gene disruption using CRISPR-Cas9 and have utilized both embryo injections and a newly developed ReMOT Control (Receptor-mediated Ovary Transduction of Cargo) strategy for delivery of gene-editing reagents. In addition, we used the PacBio long reads, 10X genomics linked reads along with chromatin capture HiC technique to achieve chromosomal level assembly of tick genome. We successfully assembled the genome in 15 scaffolds corresponding to 14 autosomes and X and Y. The availability of near complete *I. scapularis* genome along with gene-editing tools that we developed will advance our knowledge of biology of this and related tick species.

Annotating insect regulatory genomes

Hasiba Asma[1], Ellen Tieke[2], Kevin Deem[2], Yoshinori Tomoyasu[2], and Marc S. Halfon[1,3,4]
[1]University at Buffalo; [2]Miami University; [3]NYS Center of Excellence in Bioinformatics and Life Sciences; [4]Roswell Park Comprehensive Cancer Center

Our work is focused on the rapid and inexpensive discovery and annotation of gene regulatory sequences in a wide range of sequenced insect species. Regulatory sequences-e.g. enhancers-play an essential role in controlling spatial and temporal gene expression. Variation in enhancer sequences is a driving force of speciation, making it critical to understand the mechanisms underlying enhancer evolution. Earlier attempts to study regulatory sequence evolution have been constrained in scope due the limited number of known insect enhancers outside of the well-studied *Drosophila melanogaster*. Although a growing number of insect species have had their genome sequenced (476 sequenced species), there are few known enhancers, and even fewer still from a common locus across many species. We are engaged in a systematic attempt to annotate large numbers of insect genomes, and in so doing, to collate groups of potentially homologous enhancers for further study. For this purpose, we previously developed SCRMshaw (for Supervised Cis-Regulatory Module Discovery), an effective method for computational enhancer discovery. We first create a training set of known enhancer sequences, using the REDfly database, to train SCRMshaw to guide its search for other enhancers with related functions. Due to the presence of deep enhancer homologies in distantly-related insect species, SCRMshaw, when trained on *Drosophila* sequences, can also discover enhancers in a cross-species fashion in genomes as far diverged as the 345 Ma honeybee genome, with a ~75% true-positive rate. We aim to predict enhancers in 60 or more of species with an acceptable quality of assembled genomes, and make these data publicly available in a user-friendly and accessible fashion. To date, we have successfully predicted enhancers in 26 species from orders Diptera, Hymenoptera, Coleoptera, Lepidoptera, and Hemiptera. We have also improved our analysis pipeline to accurately match genes with their Drosophila orthologs. Leveraging all of these data, we selected and experimentally validated 9 of our enhancer hits from 3 different species, using transgenic fly models. Empirical testing of these cross species SCRMshaw predictions revealed 77% (7/9) had expected regulatory activity and 100% were functional enhancers in transgenic flies. An additional 10 enhancers are currently undergoing in-vivo validation. As we assemble sets of orthologous enhancers, we expect to be able to trace their evolutionary history with unprecedented molecular details.

Validation of adult mosquito population size estimation with close-kin mark-recapture (CKMR) and amplicon sequencing

Anita Lerch[1], Scott T. Small[1], T. Alex Perkins[1], and Nora J. Besansky[1]
[1]University of Notre Dame, Department of Biological Sciences, Notre Dame, IN 46556

Background: Knowing the adult mosquito census size is essential to plan future release of genetically modified mosquitoes to combat the transmission of Malaria. A novel approach to estimate population size provides close-kin mark-recapture (CKMR) method combined with amplicon sequencing genotyping.

Method: To validate the CKMR method for mosquitos, we used SLiM v3.0 to develop an agent-based mosquito model simulating the life cycle of individual mosquitoes, as well as recording all mating and genomic recombination events. Micro-haplotypes were extracted from SLiM tree-sequence file to estimate discriminatory power of different amplicon sequencing panels, and to estimate accuracy of parent-offspring pairs kinship inference with the R package CKMRsim. The mosquito census size was estimated with CKMR likelihood functions adapted for mosquitos and Metropolis-Hastings sampling.

Results: We simulated a mosquito population with a median size of 441 (CI 95% 320-617) adult females and sampled 35 (CI 95% 25-43) adult mosquitos lethally every day for a total of 21 days. Sensitivity analysis showed that 300 markers with an approximate expected heterozygosity of 0.54 per marker provided discriminatory power to differentiate parent-offspring pairs from unrelated pairs with 0.001 false negative rates and 8.69x10-17 false positive rates. However, discriminatory power between parent-offspring and sibling pairs was low due to physical linkage between genotyping markers, resulting in false-positive inferred parent-offspring pairs. Estimation of female adult population size with true observed parent-offspring pairs was 400 (CI 95% 333-485) and 313 (CI 95% 267-373) with inferred parent-offspring pairs.

Conclusion: Estimation of mosquito census size is possible with CKMR method. The adult population size was underestimated due to two factors: First, frequent lethal sampling resulted in depletion of natural mosquito population size and led to an overall greater mortality rate for adult mosquitoes. Second, daily and intensive sampling increased the probability of sampling siblings and consequently led to an increase of false positive parent-offspring pairs.

MICROBIOME

Mi casa es su casa: how an intracellular symbiont manipulates host biology

Irene Newton
Department of Biology, Indiana University

Wolbachia pipientis is the most prevalent intracellular infection on the planet and causes a myriad of different host phenotypes ranging from protection from viral pathogens to reproductive manipulations. *Wolbachia* bacteria are found in 40-60% of insect species and are primarily maternally transmitted. How do these microbes colonize new insect hosts? What tools do they use to alter host biology? I will present data from my lab – focused on *Drosophila melanogaster* - identifying the molecular toolkit for *Wolbachia*-host interaction

and host pathways that are impinged upon by the bacterium. I will also present our data showing how host cellular changes induced by *Wolbachia* alter RNA virus replication in the host cell.

Identification of viral transcripts in RNA-seq datasets from bees, wasps, mites and ants

Katherine Brown[1], Andrew E. Firth[1]
[1]Division of Virology, Department of Pathology, University of Cambridge

Many honey bee colonies suffer large losses due to colony collapse disorder. This phenomenon, which has dramatically increased in frequency since 2006, has led to widespread efforts in sequencing honey bee pathogens, including RNA viruses such as deformed wing virus. However, honey bees coexist with a number of other arthropods, whose viruses are less thoroughly characterised. Many viruses currently classified as honey bee pathogens may therefore have a much wider host range. In particular, ants, which like bees are members of the Hymenoptera order, often coexist with bees and the two groups have previously been shown to exchange viruses. Parasitism by *Varroa* mites, known to act as effective vectors for a number of RNA viruses, is also almost ubiquitous amongst honey bees, but relatively little is known about viruses endemic to mites. We have previously demonstrated that it is possible to detect and characterise viral RNA in publicly available RNA-seq datasets. There are over 3,000 such datasets for diverse Hymenoptera and mite species. We have therefore developed a computational pipeline to identify viral transcripts in these datasets. This pipeline performs quality control, removes low complexity reads and reads generated from host RNA and various known contaminants, assembles the remaining reads into transcripts and detects the presence of regions with homology to known RNA viruses. Over 20,000 putative viral fragments have been identified in these datasets, forming almost 700 clusters representing potential virus species. Phylogenetic analysis of these clusters has revealed both previously unknown viruses and known viruses in unexpected hosts. We have also developed a bioinformatics tool, CIAlign, used to clean and visualise multiple sequence alignments, to mitigate issues encountered while developing this pipeline.

Chromosomal-level assembly of *Bactericera cockerelli* reveals rampant gene family expansions and duplications of horizontally transferred genes that impact insect-microbe-plant-interactions

Younghwan Kwak[1], Jacob A. Argandona[1], Patrick H. Degnan[2*], and **Allison K. Hansen**[1*]

[1]Department of Entomology, University of California, Riverside, 900 University Ave Riverside, CA 92521 USA [2]Department of Microbiology and Plant Pathology, University of California, Riverside, 900 University Ave Riverside, CA 92521 USA

Lineage specific expansions and gene duplications are some of the most important sources of evolutionary novelty in eukaryotes. Although not as prevalent in eukaryotes compared to bacteria, horizontal gene transfer events can also result in key adaptations for insects, especially for those involved in insect-microbe interactions. In this study we assemble the first chromosomal assembly of the psyllid *Bactericera cockerelli* and reveal that the *B. cockerelli* genome has experienced significantly more gene expansion events compared to other Hemipteran representatives with fully sequenced genomes. We also reveal that *B. cockerellia's* genome

is the largest psyllid genome (567 Mb) sequenced to date and is ~15% larger than the other two psyllid species genomes sequenced (*Pachypsylla venusta* and *Diaphorina citri*). Structurally, *B. cockerelli* appears to have an additional chromosome compared to the distantly related psyllid species *P. venusta* due to a previous chromosomal fission or fusion event. The increase in genome size and dynamic nature of the *B. cockerelli* genome may largely be contributed to the widespread expansion of type I and type II repeat elements that are rampant across all of *B. cockerellia's* chromosomes. These repeat elements are distributed near equally in both euchromatic and heterochromatic regions. Furthermore, significant gene family expansions and gene duplications were uncovered for genes that are expected to be important in its adaptation to insect-plant and microbe interactions, which include transcription factors, proteases, odorant receptors, and horizontally transferred genes that are involved in the nutritional symbioses with their long-term nutritional endosymbiont *Carsonella*.

Overabundance of *Asaia* and *Serratia* bacteria is associated with deltamethrin insecticide susceptibility in *Anopheles coluzzii* from Agboville, Côte d'Ivoire

Bethanie Pelloquin[1,2], Mojca Kristan[1], Constant Edi[3], Anne Meiwald[1], Emma Clark[1], Claire L. Jeffries[1], Thomas Walker[1], Nsa Dada[4,5,6], and Louisa A. Messenger[1]
[1]Department of Disease Control, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; [2]School of Tropical Medicine and Global Health, University of Nagasaki, Nagasaki, Japan; [3]Centre Suisse de Recherche Scientifique en Côte d'Ivoire, Abidjan, Côte d'Ivoire; [4]Faculty of Science and Technology, Norwegian University of Life Sciences, Aas, Norway; [5]Public Health and Epidemiology Department, Nigerian Institute of Medical Research, Lagos, Nigeria [6]Tropical Infectious Disease Research Center, University of Abomey-Calavi, Cotonou, Benin

Insecticide resistance among mosquito species is now a pervasive phenomenon that threatens to jeopardize global malaria vector control efforts. Evidence of links between the mosquito microbiota and insecticide resistance is emerging, with significant enrichment of insecticide degrading bacteria and enzymes in resistant populations. Using 16S rRNA amplicon sequencing, we characterized and compared the microbiota of *Anopheles coluzzii* in relation to their deltamethrin resistance and exposure profiles. Comparisons between 2- and 3-day-old deltamethrin-resistant and -susceptible mosquitoes demonstrated significant differences in microbiota diversity. *Ochrobactrum*, *Lysinibacillus*, and *Stenotrophomonas* genera, each of which comprised insecticide-degrading species, were significantly enriched in resistant mosquitoes. Susceptible mosquitoes had a significant reduction in alpha diversity compared to resistant individuals, with *Asaia* and *Serratia* dominating microbial profiles. There was no significant difference in deltamethrin-exposed and -unexposed 5- to 6-day-old individuals, suggesting that insecticide exposure had minimal impact on microbial composition. *Serratia* and *Asaia* were also dominant in 5- to 6-day-old mosquitoes, which had reduced microbial diversity compared to 2- to 3-day-old mosquitoes. Our findings revealed significant alterations of *A. coluzzii* microbiota associated with deltamethrin resistance, highlighting the potential for identification of novel microbial markers for insecticide resistance surveillance. qPCR detection of *Serratia* and *Asaia* was consistent with 16S rRNA sequencing, suggesting that population-level field screening of bacterial microbiota may be feasibly integrated into wider resistance monitoring, if reliable and reproducible markers associated with phenotype can be identified.

POSTER PRESENTATIONS

I5K/EMERGING GENOMES

1. *Schistocerca* (Orthoptera: Acrididae) as a model clade for studying phenotypic plasticity from a genomic perspective

Hojun Song[1,5], Anna Childers[2,5], Olga Dudchenko[3,5], Scott Geib[2], Sheina Sim[2], Tyler Simmonds[2], Amanda Stahlke[2,5], Maeva Techer[1,5], Stephen Richards[3,5], Erez Lieberman Aiden[3,5], and Fabrizio Gabbiani[4,5]
[1]Department of Entomology, Texas A&M University, College Station, TX, USA; [2]United States Department of Agriculture, Agricultural Research Servic;e [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [4]Department of Neuroscience, Baylor College of Medicine, Houston, TX, USA; [5]NSF-BII Behavioral Plasticity Research Institute

The genus *Schistocerca* (Orthoptera: Acrididae) includes some of the most devastating locust species in the world, including the desert locust (*S. gregaria*), the Central American locust (*S. piceifrons*), and the South American locust (*S. cancellata*). These locust species show an extreme form of density-dependent phenotypic plasticity in which cryptic and shy individuals, known as the solitarious phase, can transform into conspicuous and gregarious individuals, known as the gregarious phase, in response to changes in local population density. In fact, this "locust phase polyphenism" is what makes the locusts distinctly different from regular grasshoppers. Intriguingly, *Schistocerca* includes 45 species, most of which are non-swarming sedentary grasshopper species, and phylogenetic studies have shown that the locust species do not form a monophyletic group, suggesting that locust phase polyphenism has evolved multiple times in the genus. Furthermore, recent experimental studies have indicated that some of the non-swarming grasshopper species show reduced density-dependent phenotypic plasticity, suggesting that *Schistocerca* as a whole is an exciting model clade that can be used to study how phenotypic plasticity has evolved as species diverge. Until now, it has been very challenging to study these insects from a genomic perspective because of their large genome sizes (up to 9.1 Gb), which are the largest among insects. Recently, however, we launched the Behavioral Plasticity Research Institute (BPRI) supported by the National Science Foundation Biology Integration Institute (NSF-BII) program, which allowed us to produce high-quality chromosome-length assemblies of six Schistocerca species, including three locust and three non-swarming grasshopper species. These genomes were generated using PacBio HiFi and Hi-C technologies with a target coverage of 30x and 15x, respectively. Our assemblies are highly contiguous (contig N50 ranging from 27.4 to 74.2 Mb and scaffold N50 ranging from 791.2 to 854.0 Mb) and consistent with known estimates of chromosome count (11 autosomal pairs + sex chromosome) for these species. In this presentation, we introduce these new genomes as well as other omics approaches that we are currently pursuing, including transcriptomics, epigenomics, and single-cell genomics. We also introduce the overall vision and research activities of the BPRI, and make a strong case for why *Schistocerca* is an excellent model clade for biological integration.

2. i5k in the landscape of global, regional, and national BioGenome initiatives

Robert M. Waterhouse
Department of Ecology and Evolution, University of Lausanne, and the Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland

Arthropod genomes are critical for performing cutting-edge entomological research, from gaining key insights into basic animal biology, to finding new ways to limit the effects of damaging species or protect threatened ones, and to understand the evolution of arthropod diversity on Earth. The i5k initiative to coordinate the sequencing, assembly, annotation, and analysis of 5,000 arthropod genomes was officially launched just over a decade ago (Robinson et al. 2011). The i5k pilot project formed a natural focal point for community engagement, culminating with the production of genomic resources for 28 species presented collectively in the analyses of Gene Content Evolution in the Arthropods (Thomas *et al.* 2020). In parallel, other BioGenome projects of varied taxonomic or geographic scope have been launched that have clear synergies with the overarching goals of the i5k initiative. The umbrella Earth BioGenome project (EBP, www.earthbiogenome.org) aims to coordinate the sequencing and characterisation of the genomes of all of Earth's eukaryotic biodiversity. With a network of networks model the EBP connects BioGenome initiatives such as the Africa BioGenome Project (https://africanbiogenome.org), the Ag100 Pest Initiative of the USDA (http://i5k.github.io/ag100pest), the Darwin Tree of Life project (DToL, www.darwintreeoflife.org), and the European Reference Genome Atlas (ERGA, www.erga-biodiversity.eu) initiative. This landscape of BioGenome Initiatives can seem difficult to navigate, particularly for newcomers to the i5k Arthropod Genomics Community. Here we aim to provide an introductory map that outlines ongoing efforts most relevant to the Arthropod Genomics Community as well as highlighting the tools and resources being developed to overcome scientific and organisational challenges.

3. Targeted sequencing detects candidate resistance alleles to *Bacillus thuringiensis* insecticidal proteins in invasive *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Peter Tandy[1], Kurt Lamour[1], Rodney Nagoshi[2], Caroline Placidi de Bortoli[1], **Scott Emrich[3]**, and Juan Luis Jurat-Fuentes[1]
[1]Department of Entomology and Plant Pathology, University of Tennessee, USA; [2]Center for Medical, Agricultural and Veterinary Entomology, United States Department of Agriculture-Agricultural Research Service, USA; [3]Department of Electrical Engineering and Computer Science, University of Tennessee, USA

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a highly polyphagous pest native to the tropical Americas that in the last 5 years has spread to become a global superpest threatening food and fiber production. Transgenic crops producing insecticidal Cry and Vip3Aa proteins from the bacterium *Bacillus thuringiensis* (Bt) have been used for effective control of this pest in North and South America. Evolution of practical resistance to these Bt crops represents the greatest threat to sustainability of this technology and its potential use in controlling *S. frugiperda*. Monitoring for resistance is vital to management approaches intended to delay resistance to Bt crops in *S. frugiperda*. Current monitoring efforts focus on bioassays with *S. frugiperda* larvae derived from field-collected parents. DNA-based methods present higher sensitivity and cost-effectiveness compared to current bioassay-based resistance monitoring. Targeted sequencing of the

SfABCC2 gene linked to practical resistance against Cry1F corn in Puerto Rico confirmed high frequency of a known resistance allele in this population and provided novel candidate resistance alleles. In this study, we performed targeted SfABCC2 sequencing to detect known and candidate resistance alleles in field-collected *S. frugiperda* from continental USA, Africa (Ghana, Togo and South Africa) and Southeast Asia (Myanmar). Results identify additional candidate resistance alleles in the invasive *S. frugiperda* range but did not detect the previously known allele outside the Caribbean. Conservation of some of these alleles among samples from diverse locations contributes to our understanding of *S. frugiperda* spread.

4. Overcoming scientific IT infrastructure problems for the creation, storage, and use of a large number of phased insect genomes: A case study using the two-lined spittlebug

David Molik[1], Tyler Simmonds[1], and Scott Geib[1]
[1]USDA ARS, Pacific Basin Agricultural Research Center, Tropical Crop and Commodity Protection Research

We utilize the Two-Lined Spittlebug, a tropical grazeland insect pest as an example of the use and implementation of the Only The Best (otb) pipeline and project setup. As a tropical grazeland insect pest Two Lined spittle bug is a of economic importance to Hawaiian Ranchers. A phased HiC/HiFi genome can be used in the estimation of population structure where an understanding of specific allele information might lead to Genomic based Insect Pest Management targets; Broadly, there are several applications for a large quantity of arthropod genomes, but the continuous creation of new genomes creates several data management problems. We introduced a new HiC/HiFi phased genomics assembly pipeline, otb, to reduce the amount of time spent organizing data, installing, and calibrating bioinformatic tools, and running analysis. We also introduce several scripts to move and label data for archiving. Implementing this pipeline and environment allowed us to reduce the amount of time to produce a usable genome. Careful implementation of data management and data standardization further reduced the amount of effort needed for the project team in the creation of genomes. otb is written in nextflow, utilized, and accessed through bash scripting which implements a singularity environment, and conducts software and environment checking to debug problems and ensure operation. https://github.com/molikd/otb.

5. The AgBioData Research Coordination Network: Ensuring FAIR agricultural data through community-based standards

Annarita Marrano[1], Darwin Campbell[2], Jaqueline Campbell[3], Ethalinda Cannon[3], Laurel Cooper[4], Lisa Harper[3], Eva Huala[1], Sook Jung[5], Sunita Kumari[6], Sushma Naithani[4], **Monica Poelchau**[7], Leonore Reiser[1], Meg Staton[8], Marcela K.Tello-Ruiz[6]
[1]Phoenix Bioinformatic;, [2]Iowa State University; [3]USDA-ARS CICGR; [4]Oregon State University; [5]Washington State University, Pullman; [6]Cold Spring Harbor Laboratory; [7]USDA-ARS NAL; [8]University of Tennessee, Knoxville

With the increasing volume of data generated in agriculture, developing guidelines for appropriate data sharing and management is essential. Founded in 2015, the AgBioData Consortium aims to identify the current major issues in data curation and management and ensure more Findable, Accessible, Interoperable, and Reusable (FAIR) data. In 2021 we received funding from the National Science Foundation for a

Research Coordination Network grant, whose main goals are: (1) make recommendations and implementation plans for FAIR data in agriculture; (2) expand the AgBioData network by recruiting key stakeholders in agricultural research; (3) provide educational material to train researchers on FAIR data sharing; and (4) develop a roadmap for a sustainable genomic, genetic and breeding (GGB) Database Ecosystem. We have started by launching nine working groups covering different aspects of data management in agriculture and recruitment. We also plan new groups focused on sharing and archiving new types of data, such as those generated with high-throughput phenotyping platforms. While the majority of AgBioData member databases and participants come from the plant genetics, genomics and breeding community, insect genomics databases and researchers are also welcome and needed to collectively identify best data management practices for FAIR genomics data. We are welcoming new members with expertise in the field. If you are interested in joining, please visit our website (https://www.agbiodata.org).

6. USDA-ARS's Ag100Pest Initiative: Genomic resources for next-generation pest control

Anna K. Childers[1] on behalf of the Ag100Pest Team
[1]USDA, Agricultural Research Service, Bee Research Laboratory, Beltsville, MD, USA

Arthropod pests annually damage of millions of dollars worth of US agricultural commodities including field crops, livestock, bees, trees, and stored products. In addition, some foreign pest species are considered potential invasive threats to US agriculture. Genomic resources are needed to enable molecular research into the biology, physiology, and evolution of pest species to advance novel arthropod and agricultural pest management research. The USDA Agricultural Research Service's (USDA-ARS) Ag100Pest Initiative (http://i5k.github.io/ag100pest) began with a mission to generate reference-quality genome assemblies and annotations for the top 100 US arthropod agricultural pests. Leveraging USDA-ARS's unique expertise in both arthropod pest management and genomics research the project has been a success. The goal has now expanded to over 175 projects, representing 168 species across 9 arthropod orders and 57 families. For some species, sequencing of both sexes or multiple strains is being conducted. PacBio long-read sequencing from a single specimen is complete for over 110 projects and dozens of Hi-C scaffolded assemblies have already been submitted to NCBI. These chromosome-scale assemblies are advancing the sequencing goals of both the i5K initiative and the Earth BioGenome Project. More importantly however, these assemblies are ensuring researchers have the genomic resources they need to advance innovative solutions to arthropod pest challenges while protecting beneficial insects and ecosystems.

STRUCTURAL EVOLUTION

7. Discovery of large chromosomal inversions in the genome of *Aedes aegypti* across the tropics

Jiangtao Liang[1], Andrey Yurchenko[1], Varvara Lukyanchikova[1], Ilya Brusentsov[2], Noah Rose[3], Zhijian Tu[4], Carolyn McBride[3], and Maria Sharakhova[1,2]
[1]Department of Entomology and Fralin Life Science Institute, Virginia Polytechnic and State University, Blacksburg, USA; [2]Laboratory of Cell Differentiation, the Federal Research Center, Institute of Cytology and Genetics, Novosibirsk, Russia; [3]Department of Ecology and Evolutionary Biology, Princeton

University, Princeton, USA. [4]Department of Biochemistry and Fralin Life Science Institute, Virginia Polytechnic and State University, Blacksburg, USA

Polymorphic chromosomal inversions have been shown to be associated with adaptations and traits relevant to malaria transmission in anopheline mosquitoes. However, the discovery of chromosomal inversions in aedini mosquitoes has been challenging because of the absence of high-quality polytene chromosomes. In this study, we applied a Hi-C approach to 22 strains of arbovirus vector *Aedes aegypti* from 13 countries across the tropics. Totally, 20 multi-megabase chromosomal inversions with sizes varying from 1.5 to 55 Mb were found. The presence of inversions was validated by fluorescence in situ hybridization in mitotic chromosomes. Interestingly, inversions found in *Ae. aegypti* were not distributed along chromosomes evenly and were more abundant in 1q and 3p arms, which are homologous to the inversion-rich 2R arm in the malaria vector *Anopheles gambiae*. Inversions were more abundant in populations from Western Africa where the highest number of them was observed. Our results suggest an existence of a large pool of structural variations in the *Ae. aegypti* genome potentially involved in the adaptation and pathogenesis of this mosquito.

8. Paternal genome elimination creates contrasting evolutionary patterns in male and female citrus mealybugs

Andrew J. Mongue and Laura Ross
Institute of Evolutionary Biology, University of Edinburgh, UK

Mealybugs are a uniquely interesting group of insects, both as crop pests and as exhibitors of a strange form of reproduction known as Paternal Genome Elimination (PGE). Males of this system are functionally haploid, with their father's genetic contribution transcriptionally repressed, and can only pass on their maternally inherited genes to offspring. This unusual method of reproduction should create drastically different selective pressures for males and females, but has never been studied in a population genomic framework. For the first time, we have generated whole genome resequencing data for multiple wild populations of *Planococcus citri*, the citrus mealybug as well as sex- and stage-specific RNA sequencing. We show that adult males and females have incredible sexual dimorphism in gene expression and rates of adaptive evolution strongly differ for genes expressed in male- and female-specific patterns. Furthermore, comparisons between populations offer insights on the spread of these globally distributed pest insects, making these data and results valuable in both basic and applied contexts.

VECTOR GENOMICS

9. Evolutionary characterization of innate immune related genes in six *Glossina* species

Karega Pauline[1], *Robert Waterhouse[2], Rosaline Macharia[1] and Dan Masiga[3]*
[1]University of Nairobi; [2]University of Lausanne; [3]International Centre for Insect Physiology and Ecology

Tsetse flies are vectors of trypanosomes that cause Animal and Human African trypanosomiasis. Trypanosomiasis has been implicated in losses of up to 4.75 billion US dollars in the agricultural and economic sectors. Tsetse immunity plays a role in the vector competence of *Glossina* species, with presumably different environmental and microbial exposures leading to evolutionary changes in their repertoires of immune genes. Advancement in sequencing technologies has made possible the sequencing of

whole genomes of various insect species including tsetse flies, facilitating comparative genomics studies of dipteran immune-related genes. Such initial analyses have revealed gene family expansions, contractions and gene losses in *Glossina* relative to *Musca domestica* and *Drosophila melanogaster*. However, current annotated gene models from six *Glossina* genomes require careful curation and systematic annotation for comprehensive downstream analysis. This project therefore proposed to evaluate a bioinformatics workflow that automates the process of immune gene identification and signature characterization, following manual annotation. The objectives of this study included to characterize evolutionary features of immune related genes in tsetse flies, determine the timings of gains and losses of immune related genes on the *Glossina* phylogeny and to evaluate a workflow for automated identification and characterization. Using the *Glossina morsitans morsitans* genome as a reference, manual curation of selected immune-related genes was first performed. This allowed for accurate gene prediction in the other species based on the high-quality curated gene models. Subsequent phylogenetic analyses of these genes were then performed to infer orthology and paralogy, from which gene gain and loss events were identified, and sequence evolutionary rates and constraints estimated. A relaxed molecular clock species phylogeny was then used to date the gene gain and loss events. These evolutionary features helped to understand the evolution of *Glossina* immunity and also shed light on the evolutionary relationships with other dipterans such as *Drosophila*, *Musca* and *Anopheles*. Automation of this process will also allow faster characterization of such evolutionary features of other gene families and other insects in the future. This study explored evolutionary features of immune-related genes in *Glossina*, provided accurate timings of changes in the immune repertoire across six *Glossina* species, and will develop a tool to improve future characterizations of evolutionary features in newly sequenced genomes.

10. Thrips (Thysanoptera: Paraneoptera) have an ancient relationship with their vectored tospoviruses: a genomic approach to predicting hidden vectors of disease

David A. C. Stanford-Beale and Stephen L. Cameron
Department of Entomology, Purdue University, West Lafayette, Indiana, 47907 USA

Thrips, Thysanoptera (Paraneoptera), are an enigmatic order of minute insects that vector the damaging group of crop plant viruses, tospoviruses (Tospoviridae). Investigating the origins of tospovirus vectoring in thrips has previously been hampered by the lack of support for early relationships in both the thrips and tospovirus phylogenies. A candidate gene for endoCP-GN, a possible tospovirus binding cuticle protein, has been described in the *Frankliniella occidentalis* genome. Whether or not this gene is found in other taxa was previously unknown. Using a cophylogenetic and genomic approach, we provide evidence for an ancient relationship between thrips and tospoviruses (~121 m.y.a.). We examine the cophylogeny using four different evolutionary scenarios, approximate to hypotheses present in the literature, and provide comment on the evolutionary history between thrips and their tospoviruses. We confirm the presence of assembled endoCP-GN homologs across representatives of Thripidae. We predict that tospovirus vectoring, and the potential to vector can be inferred from assembled sequences and call for the field to concentrate efforts on non-pest species of thrips that may be reservoir vectors of tospoviruses.

11. Validation of adult mosquito population size estimation with close-kin mark-recapture (CKMR) and amplicon sequencing

Anita Lerch[1], Scott T. Small[1], T. Alex Perkins[1], and Nora J. Besansky[1]
[1]University of Notre Dame, Department of Biological Sciences, Notre Dame, IN 46556

Background: Knowing the adult mosquito census size is essential to plan future release of genetically modified mosquitoes to combat the transmission of Malaria. A novel approach to estimate population size provides close-kin mark-recapture (CKMR) method combined with amplicon sequencing genotyping.

Method: To validate the CKMR method for mosquitos, we used SLiM v3.0 to develop an agent-based mosquito model simulating the life cycle of individual mosquitoes, as well as recording all mating and genomic recombination events. Micro-haplotypes were extracted from SLiM tree-sequence file to estimate discriminatory power of different amplicon sequencing panels, and to estimate accuracy of parent-offspring pairs kinship inference with the R package CKMRsim. The mosquito census size was estimated with CKMR likelihood functions adapted for mosquitos and Metropolis-Hastings sampling.

Results: We simulated a mosquito population with a median size of 441 (CI 95% 320-617) adult females and sampled 35 (CI 95% 25-43) adult mosquitos lethally every day for a total of 21 days. Sensitivity analysis showed that 300 markers with an approximate expected heterozygosity of 0.54 per marker provided discriminatory power to differentiate parent-offspring pairs from unrelated pairs with 0.001 false negative rates and 8.69x10-17 false positive rates. However, discriminatory power between parent-offspring and sibling pairs was low due to physical linkage between genotyping markers, resulting in false-positive inferred parent-offspring pairs. Estimation of female adult population size with true observed parent-offspring pairs was 400 (CI 95% 333-485) and 313 (CI 95% 267-373) with inferred parent-offspring pairs.

Conclusion: Estimation of mosquito census size is possible with CKMR method. The adult population size was underestimated due to two factors: First, frequent lethal sampling resulted in depletion of natural mosquito population size and led to an overall greater mortality rate for adult mosquitoes. Second, daily and intensive sampling increased the probability of sampling siblings and consequently led to an increase of false positive parent-offspring pairs.

12. Population genomics of "Apple Proliferation" disease transmissibility by apple psyllids and their endosymbionts

James M. Howie[1*], Erika Corretto[2], Jessica Dittmer[2,3], Liliya Serbina[2], Lino Ometto[4], Omar Rota-Stabelli[5], Christian Stauffer[1], and Hannes Schuler[2]
[1]Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), University of Natural Resources and Life Sciences Vienna, 1190 Vienna, Austria; [2]Faculty of Science and Technology, Free University of Bozen-Bolzano, University Square 1, 39100, Bolzano, Italy; [3] EmerSys Research Group, IRHS UMR 1345, INRAe Pays de la Loire Center, 42 rue Georges Morel, 49070 Beaucouzé, France; [4]Department of Biology and Biotechnology, University of Parvia, Pariva, Italy; [5] Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all'Adige, Italy

Apple proliferation (AP) is a chronic disease of apple trees caused by the bacteria *Candidatus Phytoplasma mali*, which is primarily transmitted by two phloem feeding psyllid vectors, *Cacopsylla picta* and *C.*

melanoneura. The extent of AP transmission arises from a complex interaction between *Ca. P. mali*, the psyllids, their primary and secondary endosymbionts, and the apple tree. Our research focuses especially on genetic variation in the psyllids and their endosymbionts associated with psyllid transmission efficiency and regional disease prevalence, based on field collections, field-laboratory experiments, and next- and third-generation sequencing. Populations of *C. picta*, the primary vector of *Ca. P. mali* across most of Europe, and *C. melanoneura*, an emerging vector in areas of North-Western Italy, were collected from North-Western and North-Eastern Italy. Isolines were established via single crossing of males with virgin females, and offspring sibling families were raised on experimental apple trees infected with *Ca. P. mali*. Quantitative PCR was used to determine disease uptake in individuals in each sibling family and, after high quality nuclear and mitochondrial psyllid and endosymbiont genomes were assembled, moderate to high coverage long- and short-read genomic sequencing of the sibling families was conducted. The resultant dataset allowed us to examine genetic differences associated with disease uptake rates after psyllids fed on infected apple trees, and with regions where co-evolution between *Ca. P. mali* and the psyllid vector was recent versus long established. Results provide insights into both the genomic structure of two key *Ca. P. mali* vectors and the population genomics of the psyllids and their endosymbionts with respect to their effects on spreading an agriculturally important bacterial disease.

13. Spiroplasma-induced changes in gene expression in the *Glossina fuscipes fuscipes* midgut

Erick Awuoche[1], Brian Weiss[1] and Serap Aksoy[1]
[1]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, Connecticut, United States of America

Tsetse flies (*Glossina* spp.) are the main vectors of socioeconomically devastating African trypanosomes. These flies also house an assortment of maternally transmitted endosymbiotic bacteria that influence their fly host's metabolic, reproductive, and immunological homeostasis. One recently discovered tsetse endosymbiont, *Spiroplasma*, heterogeneously infects wild and lab reared populations of tsetse from the *Glossina palpalis* subgroup. *Spiroplasma* resides intra and extracellularly within multiple tsetse tissues, and flies infected with the bacterium present a trypanosome refractory phenotype and reduced fecundity. The physiological mechanisms underpinning these phenotypes remain poorly understood. In this study, we utilized high-throughput RNA-sequencing technology to acquire insight into how infection with *Spiroplasma* alters gene expression in the midgut of *Glossina fuscipes fuscipes* (Gff), a prolific vector of trypanosomes. We found that midguts from *Spiroplasma* infected Gff exhibited significantly decreased expression of genes associated with lipid biosynthesis and amino acid metabolism. Conversely, *Spiroplasma* induced the expression of several genes that encode proteins involved in the production of reactive oxygen species. *Spiroplasma* did not impact the expression of genes involved in canonical immune pathways, suggesting that the trypanosome refractory phenotype presented by *Spiroplasma* infected Gff may reflect competition between the fly and parasite for limited nutrients. Collectively, our results suggest that tsetse responds to *Spiroplasma* infection by down-regulating the production of lipids and amino acids in an effort to modulate bacterial density. This response may also have the 'side effects' of inhibiting metabolically costly reproductive processes and reducing the ability of trypanosomes to complete their developmental cycle within their obligate tsetse vector.

14. Populations of *Aedes aegypti* in Colombia Shed Light on Gene Flow Patterns in the Americas

R. Rebecca Love[1], Rafael Vivero[2], Daniel R. Matute[2], and Daniel R. Schrider[1]
[1]Department of Genetics, School of Medicine, University of North Carolina at Chapel Hill; [2]Universidad Nacional de Colombia, Sede MedellÃn; [3]Department of Biology, College of Arts and Sciences, University of North Carolina at Chapel Hill

Aedes aegypti is a globally invasive vector of the pathogens causing human diseases such as dengue, yellow fever, Chikungunya, and Zika virus. It is highly anthropophilic, and often oviposits in manmade containers. This propensity for human proximity means Ae. aegypti is frequently transported by anthropogenic means, increasing opportunities for gene flow between distant populations. To assess patterns of gene flow in the Americas, as well as look for signatures of natural selection, *Ae. aegypti* were sampled from Medellín and Cali, Colombia, and sequenced with short-read sequencing. These data were combined with publicly available samples from Brazil, the United States of America, Gabon, Kenya, and Senegal. Our findings confirm reflect the heterogeneous nature of *Ae. aegypti*'s short- and long-range dispersal patterns. The sample from Medellín and the sample from Cali show clear separation; however, long-range connectivity was also seen between the Colombian and the Brazilian samples, consistent with previous findings of gene flow between northern Brazil and countries that border the Caribbean. Putative selective sweeps were identified across the genome, including at multiple loci implicated in insecticide resistance. We closely examined the well-known *Vssc* locus, linked to pyrethroid resistance. We see evidence of two long-range haplotypes in all South American and some North American specimens at this locus, one encompassing wild-type and the other encompassing resistant alleles at three single nucleotide variants associated with resistance. In other words, we see evidence of a selective sweep in this region; however, interestingly, Colombian as well as Brazilian specimens carrying wild-type alleles at the *Vssc* locus also show patterns consistent with a selective sweep, raising the possibility of previous or ongoing selection on other alleles. These findings have implications for understanding *Ae. aegypti*'s response to vector control.

15. Gene regulation by mating depends on sugar water and tissue type in female *Aedes aegypti*

Nanfack-Minkeu F[1], Poelstra J[2] and Sirot LK[1]
[1]Department of Biology, The College of Wooster, Wooster, OH 44691, USA; [2]Molecular and Cellular Imaging Center, Ohio Agricultural Research and Development Center, Ohio State University - Wooster campus, OH

Aedes aegypti is a major vector of several arboviruses leading to human mortality and morbidity in the world. *Ae. aegypti*-borne viruses can be controlled by manipulating the reproduction of vectors. Mating is a crucial step for the efficient reproduction of *Aedes* species. During mating, seminal fluid molecules and sperm are transferred from males to females and these stimuli influence female post-mating physiology and behavior. Yet, little is known about the effect of mating on gene expression in different tissues in *Ae. aegypti*. In addition, the influence of diet quality on gene expression is unexplored in major vectors of arboviruses. To fill in those gaps, short-read RNA sequencing was used to identify differential expressed genes in the lower reproductive tract (LRT), abdomen (Ab) and head/thorax (HT) of mosquitoes reared with 3% and 12% sugar water. The results revealed that at 3% sugar water, four, 408 and 415 significantly differential expressed genes (DEGs) were respectively identified in the HT, Ab and LRT at six hours post mating

(hpm). The number of DEGs dropped dramatically at 24 hpm with no gene in the HT, only three in the Ab and 112 in the LRT. The number of DEGs was also higher at 6hpm than 24 hpm in the LRT at 12% sugar water. Additionally, more genes were DE at 3% as compared to 12% sugar water. Gene ontology enrichment showed that oxidoreductase activity, RNA binding, and serine type peptidase were the most represented molecular functions respectively in the Ab, HT and LRT at 6 hpm. The LRT had the most diverse molecular functions including odorant binding, hydrolase, and hormone activities. At 3% sugar water, the most highly regulated genes included AAEL005032, AAEL003514 (Centromere/microtubule binding protein), AAEL006109 (Odorant binding protein 23) in the HT, Ab and LRT respectively. This study shows that mating induces post transcriptional changes depend on time point after mating, tissue type, and sugar concentration. Our results provide foundational knowledge for future functional analyses to identify genes and pathways involved in the post-mating behavioral and physiological changes of female mosquitoes.

16. Identification and trends of tRNA genes in Diptera genomes

Melissa Kelley[1], Lucas R. Klaus[1], Robert F. Grimaldi[1], and Joshua B. Benoit[1]
[1]University of Cincinnati

Transfer RNA (tRNA) are the carrier molecules that decode codons during protein synthesis. Similar to other types of RNA, tRNA are transcribed from genes within DNA. Chemical modifications to tRNA are required and influence the decoding of codons. In mosquitoes, tRNA modifications are sex-associated with the differences between the sexes being primarily anticodon modifications and there are distinct differences in tRNA gene copy number among species. The abundance of tRNAs is dependent on the composition of tRNA genes in the genome. For example, more abundant tRNAs typically have higher numbers of the corresponding tRNA gene. While trends of tRNA gene composition have been observed superficially across domains of life, studies of tRNA genes in mosquitoes and other arthropods are absent. Here, we sought to characterize tRNA genes and identify trends in tRNA gene numbers within the order Diptera with a focus on mosquitoes. Our data suggest blood-feeding insects have higher overall numbers of tRNA genes than non-bloodfeeding Diptera. All in all, we present the first survey of tRNA genes across an arthropod lineage and extrapolate trends based on phylogeny.

17. Population genetic structure and diversity for *Anopheles funestus* based on mitochondrial DNA markers (mtDNA-COI and mtDNA-COII) in western Kenya

Isaiah Debrah[1,2], Kevin O. Ochwedo[2], Maxwell G. Machani[3], Shirley A. Onyango[2], Daibin Zhong[4], Linda E. Amoah[5], Andrew K. Githeko[3], Yaw A. Afrane[6], and Guiyun Yan[4]
[1]West Africa Centre for Cell Biology of Infectious Pathogen, University of Ghana, Accra, Ghan;, [2]Sub-Saharan Africa International Centre of Excellence for Malaria Research, Homabay, Keny;, [3]Kenya Medical Research Institute, Kisumu, Kenya; [4]Program in Public Health, College of Health Sciences, University of California at Irvine, Irvine, CA, United States of America; [5]Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana; [6]Department of Medical Microbiology, University of Ghana Medical School, College of Health Sciences, University of Ghana, Accra, Ghana

In this study, the mitochondrial markers, cytochrome oxidase subunits I (mtDNA-COI) and II genes (mtDNA-COII) were used to assess the genetic structure and diversity of *Anopheles funestus*, a very important malaria vector in Africa, that adapt and colonize different ecological niches in western Kenya.

Adult mosquitoes were collected using mechanical aspirators in four sites (Bungoma, Port Victoria, Kombewa and Migori) across four counties in western Kenya. All samples were morphologically identified as *An. funestus* s.l. The mtDNA-COI and mtDNA-COII genes were amplified, sequenced and analyzed to identify sibling species and the genetic structure of the *Anopheles funestus* population. Hundred and Sixty (160) *An. funestus* s.l. specimens (40 from each site) were used for species identification, genetic structure and gene flow. The mtDNA-COI gene showed that *An. funestus* constitutes 96% of all the specimens identified. Other members of the *An. funestus* complex identified includes *Anopheles funestus*-like *Anopheles longipalpis*, *Anopheles parensis* and *Anopheles vaneedeni*. Both genes exhibit high haplotype diversity (COI, Hd=0.99; COII, Hd=0.98) but low nucleotide diversity (mtDNA-COI, Î =0.03; mtDNA-COII, Î =0.02). A minimal level of genetic differentiation was observed between Port Victoria and Bungoma (Fst = 0.01512, P= 0.00000), Port Victoria and Kombewa (Fst = 0.03135, P=0.00000), Migori and Kombewa (FST = 0.03568, P=0.000), Migori and Bungoma (Fst = 0.01621, P=0.01802) but no genetic differentiation between Port Victoria and Migori (FST = 0.00319, P= 0.180) and Kombewa and Bungoma (FST = 0.01121, P=0.0900).The high gene flow (Nm) was between Port Victoria and Migori (Nm=81.79) and low gene flow was observed between Migori and Kombewa (Nm=6.17). Neutrality tests suggest population expansion of *Anopheles funestus* with an excess of low-frequency variation. This is the first report on *Anopheles funestus*-like in western Kenya. No barrier to gene flow was observed in *Anopheles funestus* population. Population expansion suggests the high adaptability of this species.

18. Evolutionary profile of knockdown resistance (kdr) mutation in the malaria vectors *Anopheles gambiae* and *Anopheles coluzzii* malaria vectors in the mountainous plains of Cameroon

Nathalie Amvongo-Adjia[1,2,4], Jacob M. Riveron[3], Emmanuela L. Wirsiy[2], Winston P. Chounna Ndongmo[2], Flobert Njiokou[1,3], Samuel Wanji[2,5] and Charles S. Wondji[3,6]
[1]Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé, 1, P.O. Box 812 Yaoundé, Cameroon; [2]Research Foundation for Tropical Diseases and Environment (REFOTDE), University of Buea, P.O. Box 474 Buea, Cameroon; [3]Centre for Research in Infectious Diseases (CRID), LSTM Research Unit, P.O. Box 13591 Yaoundé, Cameroon; [4]Institute of Medical Research and Medicinal Plants Studies (IMPM), P.O. Box 13033 YaoundÃ©, Cameroon; [5]Department of Microbiology and Parasitology, University of Buea, P.O. Box 63 Buea, Cameroon. [6]Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool L35QA, UK.

Malaria control programmes across Africa and beyond are threatened by increasing insecticide resistance in the major anopheline vectors. In the *Anopheles gambiae* complex, two point-mutations (L1014F and L1014S) in the voltage-dependent sodium channel gene that confer target-site knockdown resistance (kdr) to DDT and pyrethroid insecticides, have been described across the northern sudano-sahelian and the southern forested zones of Cameroon, contrarily to an unclear kdr status in anophelines of mountainous agro-ecosystems across the Cameroon Great West domain. In order to determine the evolutionary profile of kdr alleles in *An. gambiae* and *An. coluzzii* sibling species both found in the Cameroon Great West domain, genotyping of the kdr locus on a total of 1172 individual specimen across 5 mountainous massifs, and sequencing of a 510 base pairs fragment of the downstream exon-20, were performed. Knockdown resistance 1014F allele was found to be widespread with *An. gambiae* having high frequencies compared to *An. coluzzii*. Meanwhile 1014S-kdr allele was confined in *An. gambiae* populations. The results suggest that kdr alleles may have arisen through introgression. Estimates of genetic variability provided evidence of selection acting on these alleles, particularly the 1014F which was driven to fixation. Spatial occurrence of

1014F was heterogenous, being seemingly influence by land elevation and gene flow. This study delineates the holistic distribution of kdr mutations in *An. gambiae* and *An. coluzzii* across mountainous ecosystems of Cameroon. Taking action to limit the spread of kdr alleles into mountainous landscapes would be helpful for the management and sustainability of malaria vector control.

19. Molecular evolution and epigenetics of Thysanoptera derived from the *Frankliniella fusca* genome assembly

Michael A. Catto[1], Paul E. Labadie[2], Alana Jacobson[3], George G. Kennedy[2], Rajagopalbabu Srinivasan[4], and Brendan G. Hunt[4]
[1]Department of Entomology, University of Georgia, Athens, GA 30602, USA; mac65630@uga.edu (M.A.C;) [2]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA; pelabadi@ncsu.edu (P.E.L.); gkennedy@ncsu.edu (G.G.K.); [3]Department of Entomology and Plant Pathology, Auburn University College of Agriculture, Auburn, AL 36849, USA; alj0043@auburn.edu (A.J.;) [4]Department of Entomology, University of Georgia, Griffin, GA 30223, USA

The tobacco thrips (*Frankliniella fusca*) is an agriculturally important pest that can transmit the tomato spotted wilt orthotospovirus to crops such as tobacco, peanut, and tomato. The structural and functional genomics within the order Thysanoptera has only begun to be explored, with the first and only genome previously resolved in the order being that of the western flower thrips (*Frankliniella occidentalis*). This study reports the genome assembly of *F. fusca*, the first thrips genome assembled from long-read sequence data. Analyses of protein evolution based on *F. fusca* and *F. occidentalis* orthologs revealed that around 1% of genes have a signal of positive selection when averaged across constituent codons. Consideration of genes with a transcriptional response to *F. fusca* infection by tomato spotted wilt virus revealed no consistent differences in the rate of protein evolution for such genes when compared to others, but several virus-responsive genes exhibit substitution patterns consistent with positive selection, highlighting potential interaction between protein evolution and pressures related to virus infection. Investigation of the mutational consequences of DNA methylation revealed the two thrips genomes have similar methylation patterns that are in line with expectations from other insect taxa, but that genes with homology to DNA methyltransferase 3 in each species are numerous and incomplete, a relatively unique phenomenon. The *F. fusca* genome opens the door to comparative genomic analyses of thysanopterans. Continued investigation of molecular evolution in thrips will help to identify genes critical to the biology and evolution of functions associated with insect-mediated virus transmission to plants. New insights into DNA methyltransferase evolution are also likely to come from comparative and biochemical studies of thysanopterans, given the large suite of loci with putative methyltransferase activity.

20. A chromosome-level assembly of the "Rockefeller" *Aedes aegypti* mosquito strain powers investigation of the evolution of pesticide resistance in a vector of human and livestock disease

Cera Fisher
Cornell University

Aedes aegypti is the vector of important human diseases, including Dengue Fever virus. Genomic resources are crucial in facilitating the study of *Ae. aegypti* and its interactions with its pathogens, ecological disturbances, and pesticide applications. Laboratory strains of *Ae. aegypti* were established in the early 20th

century, with the most commonly-used strain being "Rockefeller" (ROCK) (collected more than a century ago, probably in Cuba). A full-length genome assembly of another reference strain, Liverpool (LVP, was presumably collected in West Africa, although recent data suggests that may not be the case), was published in 2018, and is the canonical reference genome (AaegL5.3). However, relevant differences in gene expression between LVP and ROCK have been known for decades and there are significant genetic differences in *Ae. aegypti* from across the globe. This indicates that AaegL5.3 is likely not fully representative of the ROCK genome, presenting potential impediments to research. Here, we present a chromosomal-level assembly and annotation of the ROCK genome and a comparative characterization versus the LVP genome. We also present a genome-wide assessment of RNA-editing, a poorly understood phenomenon that may underpin "silent" insecticide resistance. Our results set the stage for a pan-genomic approach to understanding evolution and diversity within this important disease vector.

21. Curation and phylogenetic analysis of *Lutzomyia longipalpis* and *Phlebotomus papatasi* Cytochrome P450s

Jason Charamis[1,2], Panagiotis Ioannidis[2*], Charikleia Karageorgiou[2,3], Mary-Ann McDowell[4], and John Vontas[2,5]

[1]Department of Biology, University of Crete, Vassilika Vouton, 71409, Heraklion, Greece; [2]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology - Hellas, 73100, Heraklion, Greece; [3]Genomics Group - Bioinformatics and Evolutionary Biology Lab, Department of Genetics and Microbiology, Autonomous University of Barcelona, Barcelona, Spain; [4]Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA; [5]Pesticide Science Laboratory, Department of Crop Science, Agricultural University of Athens, 11855, Athens, Greece. *correspondence: panagiotis_ioannidis@imbb.forth.gr

Sand flies serve as vectors for pathogens of protozoan, bacterial and viral origin. They are involved in the transmission of many human diseases, most importantly leishmaniasis. Cytochrome P450s (CYPs) comprise a diverse gene superfamily encoding for enzymes involved in essential physiological functions, as well as in metabolism of xenobiotics, including insecticides used for sand fly control. The first genome sequencing effort of two primary sand fly vector species is currently under way. Within the frame of the Sand Fly Genome Project, we manually curated the CYP gene repertoires (CYPomes) of *Lutzomyia longipalpis* and *Phlebotomus papatasi*. We also performed phylogenetic comparisons using the well-characterized CYPome of A. gambiae as reference. Sand fly CYPs belong to the four clans that are typically found in insect genomes; mitochondrial (MITO), CYP2, CYP3, and CYP4. Sand flies have an ortholog for each of the major MITO and CYP2 clades, including the highly conserved 'Halloween' genes, which are involved in ecdysteroid metabolism. The CYP3 clan is generally associated with xenobiotic detoxification. Interestingly, both sand fly genomes have an expansion in the CYP3 clan, caused by gains in the CYP9J/9L, CYP6AG, and CYP6AK subfamilies. These expansions could reflect sand fly-specific expansions to environmental challenges posed by xenobiotics. Overall, this work describes the CYPomes of two sand fly reference species and provides insights on P450 evolution in this under-represented insect clade.

22. Expanding Ensembl Metazoa arthropods

Jorge Alvarez-Jarreta[1], Lahcen Campbell[1], Vasily Sitnik[1], Matthieu Barba[1], Disha Lodha[1], Gareth Maslen[1], Sarah Dyer[1]

[1]European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge, CB10 1SD, United Kingdom

Pests and host-vector-pathogen interactions have a huge impact on human health and agricultural production. Arthropods play a central role, ranging from ticks as vectors in the transmission of pathogens such as borrelia, to invasive pests such as the silver-leaf whitefly, which confer over 200 different plant-virus species causing the spread of diseases such as African cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) leading to large scale production losses in rural Sub-Saharan Africa. As research communities develop genomic resources to help understand and control these species, Ensembl Metazoa (http://metazoa.ensembl.org) provides an open access platform to integrate publicly available genome-scale data sets for non-vertebrate metazoa species, providing access to genome browsers, tools and comparative genomics resources. Ensembl release e106 includes 101 arthropods, of which 79 are insects and 15 are arachnids. Ensembl Metazoa has a long-running collaboration with VEuPathDB and 49 of these vector species are shared across both platforms. As new data become available for key species in agricultural, environmental or host-pathogen interaction events, we aim to expand our arthropod selection to support the scientific community. We are working to increase the amount of species not only in the current large clades such as insects and arachnids, but also in those clades with little representation to date. Within these, we are focusing on adding 23 insects and 2 arachnids in release e107, 11 new crustaceans in e108, and 17 new arthropods in e109, focusing on expanding the diversity of ants and bees. In just three releases we are planning to expand by >50% the number of arthropod genomes available in Ensembl Metazoa.

23. Differential gene expression in *Culex pipiens* mosquitoes exposed to different avian *Plasmodium* lineages.

Marta Garrigos[1], Guillem Ylla[2], Josué Martínez-de la Puente[3,4], Jordi Figuerola1[4], María José Ruiz-López,[4]
[1]Estación Biológica de Doñana – CSIC, Calle Américo Vespucio 26, E-41092 Sevilla; [2]Laboratory of Bioinformatics and Genome Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland;[3]Departamento de Parasitología, Universidad de Granada, Granada, Spain; [4]CIBER Epidemiología y Salud Pública (CIBERESP), Spain.

Avian malaria is a world-wide distributed mosquito-borne disease that affects birds and it is caused by intracellular parasites. The most widespread genus causing avian malaria is *Plasmodium*, which is mainly transmitted by *Culex sp*. mosquitoes and is an extremely diverse clade with over 50 species. This extensive genomic variation translates into different phenotypic characters, including differences in virulence. Although the fitness consequences on birds are well known, there is little information on the consequences of infection for mosquitoes. A recent study showed that in *Cx. pipiens*, *P. relictum* infections cause higher mortality than P. cathemerium infections, and the transmissibility of *P. relictum* is lower than that of *P. cathemerium*. To further understand the differences in virulence between these species, we studied how *Cx. pipiens* mosquitoes respond to infection. We carried out experimental infections and characterized the transcriptional response of *Cx. pipiens* mosquitoes fed with bird blood infected with *P. relictum* and *P. cathemerium*. We studied differential gene expression at three different time-points that correspond with three key stages of *Plasmodium* development in the mosquito vector, 24 hours post feeding, 10 days post feeding and 21 days post feeding. A total of 2,050 genes were differentially expressed, the vast majority at

24 hours post feeding. The comparison between *P. relictum* and *P. cathemerium* infected mosquitoes showed 1 257 differentially expressed genes, including genes associated with the mosquito immune response and trypsin metabolism. GO terms for biological processes metabolic regulation and microtubule processes were enriched for up-regulated genes, while down-regulated genes included nucleic acids and nucleotides metabolic processes. Molecular functions related to nucleic acid and microtubule binding were enriched in up-regulated genes in *P. relictum* infected mosquitoes compared to *P. cathemerium*. Most down-regulated enriched molecular functions were related to protein and nucleotides binding. Some of the genes differentially expressed in *P. relictum* infected mosquitoes have been shown to disrupt sporogonic development by aborting the normal development of oocysts. This suggests that *P. cathemerium* and *P. relictum* elicit a different response in the mosquito that might have an effect on the pathogen development and lead to different outcomes in transmissibility and mosquito mortality.

24. Evaluation of the immunogenic properties of *Trypanosoma cruzi* consensus enolase using a bioinformatics approach

A. Díaz-Hernández[1], M.C. González-Vázquez[1], M.L. Cedillo-Ramirez[1] and A. Carabarin-Lima[1].
[1]Posgrado en Microbiología, Centro de Investigaciones en Ciencias Microbiológicas, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Puebla, Pue., Mexico

There is currently no vaccine against American trypanosomiasis, caused by the parasite *Trypanosoma cruzi*. This is due to the genomic variation observed in the six DTU´s of *T. cruzi*. The aim of this work is to propose a consensus sequence of the enolase protein, from different strains of *T. cruzi* and evaluate mainly its immunogenic properties at the bioinformatic level. From specialized databases, 15 sequences of the enolase gene were aligned to obtain a consensus sequence, this sequence was modeled and then evaluated and validated through different bioinformatic programs to know their immunogenic potential. Finally, chimeric peptides were designed with the epitopes most representative. The results showed high immunogenic potential with six epitopes for MHC-I, seven epitopes for MHC-II, all they are highly representative of the enolase present in strains from the American continent, as well as five epitopes for B cells. Regarding computational modeling, molecular Docking with Toll-like receptors showed a high affinity and low constant of dissociation, which could lead to an innate-type immune response that helps to eliminate the parasite. In conclusion, the consensus sequence proposed for enolase is capable to provide an ideal immune response; however, the experimental evaluation of this enolase consensus and their chimeric peptides should be a high priority to develop a vaccine against Chagas disease.

25. Gene expression and metabolite changes in the American Dog Tick during pesticide exposure

Souvik Chakraborty[1], Atit Pathak[1], Kennan Oyen[1], Andrew J. Rosendale1[2], and Joshua B. Benoit[1]
[1]Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45211; [2]Biology Department, Mount St. Joseph University, Cincinnati, OH, 45233

The expansion and increased prevalence of tick-borne pathogens have become an alarming public health concern. Strategies to control ticks include a multitude of techniques, among which, targeted applications of pesticides to tick habitats are one of the most effective. The general effect of pesticides on ticks, but very little is known about the molecular response following pesticide treatments in ticks. In this study, we

examined the physiological response of American dog ticks, *Dermacentor variabilis*, to five common pesticides (amitraz, chlorpyrifos, fipronil, permethrin, and propoxur) and a repellent DEET using a combined transcriptomics and metabolomics approach. During the non-lethal doses of pesticide exposure, a varying number of transcripts were differentially expressed, and only one upregulated across all treatments. We discovered neurotransmitter actions are a major biochemical target of the pesticides in the American dog tick. Metabolomic profiling showed that acetylcholine metabolism was disrupted following exposure to organophosphate pesticides, which was attributed to reduced cholinesterase activity. In fipronil-treated ticks, alterations in 4-aminobutyrate content were detected-which was likely as this pesticide acts as a gamma aminobutyric acid (GABA) receptor antagonist and leads to excessive nervous system excitation and death in the ticks. In combination with RNA sequencing, our metabolomics study revealed the physiological consequences of pesticide stress and tolerance, revealing several novel biomolecular pathways. In particular, we discovered a breakdown of amino acids following permethrin treatment, as well as an upregulation of glutamate metabolism in amitraz treated samples. Overall, our study can help formulate efficient chemicals and develop new treatment protocols to reduce the burden of ticks.

26. Molecular characterization and transcriptomic analysis of leishmaniasis vectors in Sudan

Arwa Elaagip[1,2,3], Michael Wilson[2], and Muzamil Abdel Hamid[3]
[1]Department of Parasitology and Medical Entomology, Faculty of Medical Laboratory Sciences, University of Khartoum, Sudan; [2]Noguchi Memorial Institute for Medical Research, Ghana; [3]Institute of Endemic Diseases, University of Khartoum, Sudan

Phlebotomine sand flies are responsible for the spread of Leishmania parasites. The sand fly midgut presents a number of biological barriers the *Leishmania* parasite must circumnavigate to proliferate and develop inside the insect vector. Sand fly midgut is therefore a fundamental organ representing a key target for interruption of Leishmania development and transmission. Despite this, few molecules in the midgut have been characterized. Acquiring a better understanding of the molecules present in this organ will illuminate the potential molecular interactions occurring between the *Leishmania* and the vector. This study has been undertaken on *Phlebotomus papatasi* sand flies collected from endemic and non-endemic areas of leishmaniasis in Sudan. The RNA was extracted from bodies of wild-caught blood-fed and unfed females using the Trizol method. RNA-seq and transcriptome libraries of blood-fed and unfed sand flies were prepared using the SENSE mRNA-Seq Library Prep Kit V2. The sequence reads were uploaded to the Galaxy platform using SMART-RDA for data analysis. A variety of molecules have been obtained in high quality and data information will be deposited in an international gene data bank. Overall, *Ph. papatasi* RNAseq data can be further tested in order to explore their biological and pharmacological properties, which may enhance the development of new and improved drugs/vaccines, prevention strategies, and control policies for leishmaniasis.

27. Genetic diversity of genes involved into *Anopheles gambiae s.l'* fertility and vector competency in Sub-Saharan Africa

Fatoumata Seck[1,2], Mouhamadou Fadel Diop[1], Amadou Diallo[3], Idrissa Dieng[3], Abdoulaye Diabate[2,4], Ibrahima Dia[3] & Benoit Sessinou Assogba[2]
[1]Medical Research Council Unit The Gambia at LSHTM, Banjul, The Gambia; [2]African Centre of Excellence in Biotechnological Innovations for Vector-borne Diseases Elimination (CEA ITECH-MTV),

Université Nazi Boni, Bobo-Dioulasso, Burkina Faso; [3]Institut Pasteur de Dakar, Dakar, Senegal; [4]Institut de Recherche en Sciences de la Santé/ Centre Muraz, Bobo-Dioulasso, Burkina Faso

Malaria remains a major public health burden, primarily in Sub-Saharan Africa. Dual resistance of *Anopheles* to insecticides and *Plasmodium* to antimalaria challenge and call for the development of new control strategies toward malaria elimination. Thus, the interest of targeting a specific gene function with small molecule inhibitors to control malaria is increasing. However, such approach might not be feasible if the target gene is highly diverse. Here, we analyzed the genetic diversity of HPX15 (AGAP013327) a mating-induced heme peroxidase protein also called Immunomodulatory peroxidase which have been shown to be involved in *Anopheles* fertility and vector competency, using the sequencing data of the phase3 Ag1000G project. VCF SNPs data of the HPX15 gene (3L: 10786057-10788017) have been extracted from whole genomic data of Sub-Saharan Africa *Anopheles gambiae s.l* populations to perform a principal component analysis (PCA), calculate the fixation index of population structural differentiation (Fst) and the nucleotide diversity (Pi), and to perform Tajima's D neutrality tests and phylogenetic analysis between countries and species. The PCA, phylogenetic trees and fixation index calculation showed that *An. arabiensis* populations were slightly diverged from *An. gambiae s.s.* and *An. coluzzii* populations and their intermediates (SM) closer to *An. coluzzii* populations (Fst=0.091) than *An. gambiae* populations (Fst=0.115). Similarly, the nucleotide diversity was low in all populations with the highest Pi (less than 0.02) observed in *An. arabiensis* populations specifically collected in the eastern countries (Kenya, Tanzania, Malawi and Uganda). Within *An. gambiae* populations the highest Pi was observed in Tanzania, Uganda and Cameroon, and in Gambia and Burkina for *An. coluzzii* populations, and Kenya' for the SM populations. The Tajima's D values were in overall suggesting a balancing selection. Globally, this population genetic analysis suggested that HPX15 is likely conserved within species of the *An. gambiae* complex across sub-Saharan Africa and could be targeted with small molecule inhibitors to reduce malaria transmission. However, further analysis of the SNP effect on the proteins model structure and catalytic site might be necessary to improve the compounds designing.

ARTHROPOD OMICS

28. Assembly and characterization of W chromosome in monarch butterfly (*Danaus plexippus*)

Martina Dalikova[1], Daniel Portik[2], Sarah B. Kingan[2], Hayley Mangelson[3], Liuqi Gu[1], James R. Walters[1]
[1]Department of Ecology and Evolutionary Biology, The University of Kansas, Lawrence, KS 66045, USA; [2]Pacific Biosciences, Menlo Park, CA 94025, USA 3 Phase Genomics, Seattle, WA 98109, USA

Degenerated sex chromosomes have a precedent of being notoriously difficult to assemble. Recent advances in long-read sequencing are catalyzing increases in the number and quality of W and Y assemblies, providing novel insights concerning the content and evolution of these elusive chromosomes. Here we report a novel genome assembly for the monarch butterfly (*Danaus plexippus*), focusing on the W chromosome. This species harbors a neo-Z chromosome, arising from the fusion of the ancestral Z with an autosome. Previous cytogenetic analyses indicated a similarly large and bipartite W chromosome, suggesting the possibility of a comparable neo-W, but much ambiguity remains concerning the sequence and history of monarch W chromosome. We generated PacBio HiFi reads with Hi-C data from females to support *de novo* assemblies of maternal and paternal haplotypes using Trio binning. Putative W contigs from the maternal genome were determined based on male to female coverage and sex specific kmers. The paternal assembly and W contigs were scaffolded using Hi-C data. This produced chromosomal level

scaffolds for the Z and all autosomes, including three autosomes assembled from telomere to telomere. The W chromosome scaffold length was around 10Mbp, thus leaving about one third of this chromosome in unscaffolded contigs. While primarily composed of transposable elements (TEs), the W contains at least two protein coding genes that arose through retroposition and ectopic recombination from other chromosomes. Neither of these W-linked copies appear pseudogenized and one appears to be evolving adaptively. The prevalent repetitive content of the W chromosome are elements from the LINE and LTR retrotransposon groups. Surprisingly, the TEs on W chromosome have lower divergence compared to the rest of the genome, suggesting their ongoing accumulation. Finally, despite this novel W assembly, strong evidence for or against a neo-W chromosome origin remains elusive.

29. Genome size evolution in the diverse insect order Trichoptera

Jacqueline Heckenhauer[1,2], Paul B Frandsen[1,3,4], John S Sproul[5], Zheng Li[6], Juraj Paule[7], Amanda M Larracuente[8], Peter J Maughan[9], Michael S Barker[6], Julio V Schneider[10], Russell J Stewart[11], Steffen U Pauls[1,2,12]

[1]LOEWE Centre for Translational Biodiversity Genomics (LOEWE-TBG), Frankfurt 60325, Germany [2] Department of Terrestrial Zoology, Senckenberg Research Institute and Natural History Museum Frankfurt, Frankfurt 60325, Germany [3]Department of Plant & Wildlife Sciences, Brigham Young University, Provo, UT 84602, USA [4]Data Science Lab, Smithsonian Institution, Washington, DC 20560, USA [5]Department of Biology, University of Nebraska Omaha, Omaha, NE 68182, USA [6]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA [7]Department of Botany and Molecular Evolution, Senckenberg Research Institute and Natural History Museum Frankfurt, Frankfurt 60325, Germany [8]Department of Plant & Wildlife Sciences, Brigham Young University, Provo, UT 84602, USA [9]Department of Plant & Wildlife Sciences, Brigham Young University, Provo, UT 84602, USA [10]Department of Terrestrial Zoology, Senckenberg Research Institute and Natural History Museum Frankfurt, Frankfurt 60325, Germany [11]Department of Terrestrial Zoology, Senckenberg Research Institute and Natural History Museum Frankfurt, Frankfurt 60325, Germany [12]Institute for Insect Biotechnology, Justus-Liebig-University, Gießen 35390, Germany

Aquatic insects are underrepresented in genomic studies. This is also true for the diverse order caddisflies (Trichoptera) Trichoptera. Similar to lepidopteran caterpillars, caddisfly larvae produce silk in specially modified labial glands. In fact, their silk secretion forms the basis for their diverse case-making behavior which, in turn, may allow caddisfly larvae to exploit a wide range of ecological niches. To expand the availability of aquatic insect genomes, we generated de novo genome assemblies of 17 caddisflies covering major lineages of the order. We used these genomes to understand genome size evolution and detected a ~14-fold variation in genome size across the order. We find strong evidence that repetitive element expansions, particularly those of transposable elements (TEs), are important drivers of large caddisfly genome sizes. Using an innovative method to examine TEs associated with universal single copy orthologs (BUSCO genes), we find that TE expansions have a major impact on protein-coding gene regions, with TE-gene associations showing a linear relationship with increasing genome size. Expanded genomes preferentially evolved in caddisfly clades with a higher ecological diversity (i.e., various feeding modes, diversification in variable, less stable environments). Our findings provide a platform to test hypotheses about the potential evolutionary roles of TE activity and TE-gene associations, particularly in groups with high species, ecological, and functional diversities.

30. Investigating the unusual chromatin organization and other unique biological phenomena, in the mealybug *Maconellicoccus hirsutus* using multi-omics approach

Surbhi Kohli[1], Ankita Narang[2], Parul Gulati[1], Jayant Maini[3], and Vani Brahmachari[1]
[1]Dr.B.R.Ambedkar Center for Biomedical Research, University of Delhi, Delhi, India; [2]Departments of Medical Genetics, Psychiatry, and Physiology & Pharmacology, University of Calgary, 254 HMRB, 3330 Hospital Dr NW Calgary, AB T2N 4N1, Canada; [3]Manav Rachna Campus Rd, Gadakhor Basti Village, Sector 43, Faridabad, Haryana 121004 Manav Rachna University

Mealybugs are invasive pests with a broad host range and widespread global distribution. The diploid genome consisting five chromosomal pairs (2n=10), lacks distinct sex chromosomes. The sex determination is associated with selective inactivation and heterochromatization of paternal genome in males as a result of imprinting. Thus making, it an interesting model to study imprinting mechanisms. Mealybugs exhibit many other distinctive biological characteristics including extreme sexual dimorphism, high radioresistance, meiotic drive and nested endosymbiosis. We explored different genes contributing this unique biology of mealybug, *Maconellicoccus hirsutus* by utilizing the genome sequence and annotation generated in lab. Comparative genome analysis with other insects revealed expansion of pesticide, radiation and desiccation resistance genes conferring them better adaptation in extreme environments. Circadian pathway and some epigenetic modifier genes were absent. DNA methylation machinery in mealybugs is complete except de-novo DNA methyltransferase, *DNMT3*. We performed transcriptome sequencing and differential gene expression analysis in *M. hirsutus* males and females. The genes showing enriched expression in males and females functionally correlated with their dimorphic behaviour and morphology as well as sex specific physiology. Molecular studies in mealybugs revealed presence of Nuclease Resistant Chromatin (NRC), an unusual chromatin organization resistant to micrococcal nuclease, specifically associated with males. We performed DNA sequencing and proteomics analysis to examine this ribonucleoprotein fraction for its role in heterochromatization in mealybugs. We identified specific DNA sequence motifs and heterochromatin related proteins associated with NRC, suggesting its potential role in male specific facultative heterochromatin formation in mealybugs. Thus, utilizing high throughput genomic, transcriptomic and proteomic studies we gained interesting insights regarding mealybug biology, while data generated could serve as resource for further studies.

31. The evolution of insect visual opsin genes with specific consideration of the influence of ocelli and life history traits

Quentin Guignard
FABI, University of Pretoria

Background: Visual opsins are expressed in the compound eyes and ocelli of insects and enable light detection. Three distinct phylogenetic groups of visual opsins are found in insects, named long (LW), short (SW) and ultraviolet (UV) wavelength sensitive opsins. Recently, the LW group was found to be duplicated into the LW2b and the LW2a opsins. The expression of LW2b opsins is ocelli specific in some insects (e.g., bees, cricket, scorpion flies), but the gene was not found in other orders possessing three or less ocelli (*e.g.,* dragonflies, beetles, moths, bugs). In flies, two LW2b homologs have been characterised, with one expressed in the ocelli and the other in the compound eyes. To date, it remains unclear which evolutionary forces have driven gains and losses of LW opsins in insects. Here we take advantage of the recent rapid

increase in available sequence data (*i.e.,* from insect genomes, targeted PCR amplification, RNAseq) to characterize the phylogenetic relationships of 1000 opsin sequences in 18 orders of Insects. The resulting phylogeny discriminates between four main groups of opsins, and onto this phylogeny we mapped relevant morpho-logical and life history traits.

Results: Our results demonstrate a conserved LW2b opsin only present in insects with three ocelli. Only two groups (Brachycera and Odonata) possess more than one LW2b opsin, likely linked to their life history. In flies, we hypothesize that the duplication of the LW2b opsin occurred after the transition from aquatic to terrestrial larvae. During this transition, higher flies (Brachycera) lost a copy of the LW2a opsin, still expressed and duplicated in the compound eyes of lower flies (Nematocera). In higher flies, the LW2b opsin has been duplicated and expressed in the compound eyes while the ocelli and the LW2b opsin were lost in lower flies. In dragonflies, specialisation of flight capabilities likely drove the diversification of the LW2b visual opsins.

Conclusion: The presence of the LW2b opsin in insects possessing three ocelli suggests a role in specific flight capa- bilities (e.g., stationary flight). This study provides the most complete view of the evolution of visual opsin genes in insects yet, and provides new insight into the influence of ocelli and life history traits on opsin evolution in insects.

32. A chromosome-level genome assembly of *Daphnia pulex*

Zhiqiang Ye*, Wei Wen, Trent Bishop, and Michael Lynch
Biodesign Center for Mechanisms of Evolution, Arizona State University, Tempe, AZ 85287 USA
*Corresponding author's email: zhiqiang.yes@gmail.com

Daphnia pulex is a small crustacean that has been used in ecological, evolutionary, and population genetic studies. Despite this, its genome assembly remains fragmented, impeding chromosomal level studies. To address this, we assembled the *D. pulex* (KAP4) genome to the chromosome level using PacBio HiFi reads, with most chromosomes composed of one or two contigs. The assembly generated in this study is consistent with the genetic map created for this species, allowing us to estimate the recombination rate across each chromosome. To broaden the utility of the KAP4 assembly, we screened for markers linked with the origin of obligatory asexuality and loss of male production. Additionally, we examined markers related with *D. pulex* and *D. pulicaria* speciation. Finally, we analyzed synteny between *D. pulex* and *D. magna* and discovered that the two species' distinct chromosomal numbers are the result of two chromosome fission events. The *D. pulex* genome assembly and the markers generated in this study provided valuable resources for future research on *D. pulex*.

33. A multi-omics approach reveals contrasting population-specific genome-wide epigenetic profiles across latitude-altitude range extremes in the bumble bee *B. vosnesenskii*

Sarthok Rasique Rahman[1] and Jeffrey D. Lozier[1]
[1]Department of Biological Sciences, The University of Alabama, Tuscaloosa, Alabama, USA.

Unraveling the evolutionary and ecological processes underlying adaptation to complex biological niches through NGS based approaches allows us to determine the intraspecific diversity at genomic,

transcriptomic, and epigenetic levels of a particular species distributed across complex bioclimatic gradients and thus can improve our understanding of its potential of successful adaptive responses across a varying degree of climatic factors. Our research undertakes a multi-omics approach to assess genomic and epigenetic variability in the montane bumble bee, *B. vosnesenskii*. This widespread species is distributed across latitude and altitudinal gradients in Western North American Sierra-Cascades mountains and is an ideal model system to study the latitudinal and altitudinal effects on species evolution. We assessed the genome-level diversity in *B. vosnesenskii* by generating an SNP dataset (n= 1,162,015 sites) from whole-genome sequencing of multiple samples from northern high-elevation (Oregon) (n=8) and southern low elevation (California) sites (n=10). Our result suggests *B. vosnesenskii* is genetically homogeneous (low genome-wide FST and highly correlated nucleotide diversity) as we detected no noticeable population structure between populations. Then, we assessed the intraspecific epigenetic variation for these populations using high coverage whole genome bisulfite sequencing. We discovered evidence of intrapopulation clustering, with especially low methylation variation in high elevation sites. We also found that general methylation patterns vary substantially across genomic regions, with both highly (>50%) and sparsely methylated sites (10-50% average methylation across all samples) are disproportionately found in promoters and exons. Finally, we detected 2066 differentially methylated sites (assessed at a minimum of 10% methylation difference) between the two populations, and 87.6% of sites were hypomethylated in the high elevation (OR) population. Almost all the differentially methylated sites are also present in exons and promoters, suggesting their functional role in gene regulation. Gene Ontology (GO) analyses of genes harboring differentially methylated sites involved functional enrichment of mRNA spicing, gene transcription regulation, and cellular transport machinery. Our research findings indicate intraspecific epigenetic variation may play a crucial role in adaptation to environmental extremes by regulating niche-specific gene expression to generate regional variation for responding to environmental differences and is a possible mechanism for facilitating climate-induced future range shift processes.

34. Molecular evolution of Cytochrome P450s in Tephritidae

Jason Charamis[1,2], Melina Fotiadou[1,2], Georgia Kesisoglou[1], Panagiotis Ioannidis[1] and John Vontas[2,3]
[1]Department of Biology, University of Crete, Vassilika Vouton, 71409, Heraklion, Greece; [2]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, 73100, Heraklion, Greece; [3]Pesticide Science Laboratory, Department of Crop Science, Agricultural University of Athens, 11855, Athens, Greece

Cytochrome P450s (CYPs) comprise an enzyme superfamily with multiple functions, ranging from core physiological processes to detoxification of xenobiotics. Tephritidae is a diverse family of frugivorous flies which lay their eggs into fruits, where the emerging larvae feed and develop, until they fall into the soil and reach pupation. Due to the high adaptability and their capacity to attack economically important fruit and vegetable crops, Tephritidae are among the most notorious groups of agricultural pests worldwide. Here we describe the manual curation and phylogenetic analysis of CYP gene repertoires (CYPomes) from five tephritid species with varying numbers of larval hosts. We report that all five Tephritidae species have higher number of CYP genes compared to *Drosophila melanogaster*, mainly attributed to expansions in the *CYP6* (CYP3 clan) and *CYP12* (MITO clan) families. These expansions are localized in genomic clusters that are conserved within Tephritidae and are possibly associated with general aspects of tephritid physiology, including adaptations to the frugivorous lifestyle. Between tephritids, CYP gene diversity is mostly caused by species-specific duplications occurring in the CYP6 and CYP12 clusters. Furthermore, we investigated CYP expression across multiple developmental stages and identified sets of genes with stage-enriched

expression. Interestingly, we found that many of the CYP genes with larvae-enriched expression belong to the expanded *CYP6* and *CYP12* families. Furthermore, some of these genes commonly have larvae-enriched expression in different tephritid species. These CYPs could have a conserved role in tephritid larval growth, by contributing to detoxification of secondary metabolites and toxins present in host fruits. Overall, this study aims to identify candidate CYP genes which are involved in conserved as well as species-specific physiological adaptations in tephritid flies. In addition, this work has generated an extensive catalog of manually curated CYP genes in four Tephritidae species, thus providing a rich resource for future studies.

35. The Glassy-winged Sharpshooter genome sheds light on the molecular basis of brochosomes

Zheng Li and Nancy Moran
The University of Texas at Austin

Brochosomes are proteinous structures that are uniquely found in leafhoppers. Although the exact biological function of brochosomes remains unknown, it has been hypothesized that they might involve waterproofing, camouflage, and preventing fungal infection. Previous transcriptomic analyses have shown proteins of brochosome are produced by a few orphan gene families in leafhoppers. However, research on the molecular basis of brochosomes is hampered by the lack of a high-quality reference genome of leafhoppers. Here, we used PacBio long-read sequencing and Dovetail Omni-C technology to generate a chromosome-level genome assembly of the Glassy-Winged Sharpshooter (Homalodisca vitripennis, GWSS). We validated our genome assembly by empirically estimating the genome size using flow cytometry. In addition, we used karyotyping to determine the chromosome number, which corresponds to the number and sizes of large scaffolds. We also identified the X chromosome using relative depth of sequencing reads from males and females, generated by the i5K genome project. Using both differential gene expression in tissues producing brochsomes and proteomics of brochosomes themselves, we identified and confirmed genes and proteins that underlie brochosome production. Using our genome assembly, we investigated the chromosomal locations of these genes and found tandem duplications might play an important role in the diversification of brochosome-related genes. Thus, the high quality GWSS genome sheds light on the molecular basis of brochosomes, and will likely be useful in elucidating other aspects of GWSS biology.

36. HymenopteraMine: Improved homology and gene ontology enrichment analyses

Deborah A Triant[1], Amy T Walsh[1], Christine G Elsik[1,2,3]
[1]Division of Plant Sciences; [2]Division of Animal Sciences 3MU Institute for Data Science & Informatics, University of Missouri, Columbia, MO 65211, USA

HymenopteraMine is a data mining system of the Hymenoptera Genome Database (HGD; http://hymenopteragenome.org). It is built upon the open-source InterMine platform and integrates genome assemblies and annotation data for hymenopteran species. It enables researchers to query gene annotation data to create customized datasets that can be integrated with their own data. The current HymenopteraMine release includes 58 species consisting of 23 ants, 20 bees, 11 wasps and 4 sawflies. Researchers can use simple or comprehensive tools to search across datasets that have been collected from

external sources and computed at HGD. Data sources include genes (RefSeq, Official Gene Set), homologs (OrthoDB), Gene Ontology (Uniprot-GOA), proteins and protein domains (UniProt, InterPro), pathways (KEGG) and publications (PubMed). Additionally, to address incomplete species representation in OrthoDB, we have computed orthologs for all species using OrthoLoger, the OrthoDB pipeline and provide ortholog clusters for 14 taxonomic groups. Similarly, to augment the Uniprot-GOA data, we have computed Gene Ontologies for all species based on orthology and InterPro domains resulting in more than a 10-fold increase in the number of GO-annotated genes. The well annotated genome of *Drosophila melanogaster* from the Dipteran outgroup is included so that users can search for orthologous hymenopteran genes for pathway analysis (Reactome) and interactions (BioGRID). The different resources within HymenopteraMine are accessible through the menu bar found on every page with detailed help documentation and tutorials available. There are simple tools such as keyword searches and built-in template queries that can serve as starting points for new users. More complicated queries can be performed using genome coordinates with the Genomic Regions search tool or lists of identifiers can be uploaded and analyzed with the List Tool. More elaborate inquiries can be conducted with the powerful QueryBuilder. HymenopteraMine supports meta-analyses by cross referencing identifiers from gene sets across databases. Results can be saved for future HymenopteraMine sessions or exported in a variety of formats (GFF3, tab-delimited, Fasta, BED, JSON, XML).

37. Chromosomal-level reference genome of the moth *Heortia vitessoides* (Lepidoptera: Crambidae), a major pest of agarwood-producing trees

Sean T.S. Law[1,^,*], Wenyan Nong[1,^], Wai Lok So[1], Tobias Baril[2], Thomas Swale[3], Chi Bun Chan[4], Stephen S. Tobe[5], Zhen-peng Kai[6], William G. Bendena[7], Alexander Hayward[2], Jerome H.L. Hui1[*]
[1]School of Life Sciences, Simon F.S. Li Marine Science Laboratory, State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Hong Kong, China; [2]University of Exeter, United Kingdom; [3]Dovetail Genomics, United States of America; [4]School of Biological Science, The University of Hong Kong, Hong Kong, China; [5Department] of Cell and Systems Biology, University of Toronto, Canada; [6]School of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai, China; [7]Department of Biology, Queen's University, Canada; ^ = contributed equally; * = corresponding author: seantslaw@link.cuhk.edu.hk, jeromehui@cuhk.edu.hk The moth

Heortia vitessoides Moore (Lepidoptera: Crambidae) is a major pest of ecologically, commercially and culturally important agarwood-producing trees in the genus *Aquilaria*. In particular, *H. vitessoides* is one of the most destructive defoliating pests of the incense tree *Aquilaria sinesis*, which produces a valuable fragrant wood used as incense and in traditional Chinese medicine (Jin et al 2016). Nevertheless, a genomic resource for *H. vitessoides* is lacking. Here, we present a chromosomal-level assembly for *H. vitessoides*, consisting of a 517 megabase (Mb) genome assembly with high physical contiguity (scaffold N50 of 18.2 Mb) and high completeness (97.9% complete BUSCO score). To aid gene annotation, 8 messenger RNA transcriptomes from different developmental stages were generated, and a total of 16,421 gene models were predicted. Expansion of gene families involved in xenobiotic metabolism and development were detected, including duplications of cytosolic sulfotransferase (SULT) genes shared among lepidopterans. In addition, small RNA sequencing of 5 developmental stages of *H. vitessoides* facilitated the identification of 85 lepidopteran conserved microRNAs, 94 lineage-specific microRNAs, as well as several microRNA clusters. A large proportion of the *H. vitessoides* genome consists of repeats, with a 29.12% total genomic contribution from transposable elements, of which long interspersed nuclear elements (LINEs) are the

dominant component (17.41%). A sharp decrease in the genome-wide percentage of LINEs with lower levels of genetic distance to family consensus sequences suggests that LINE activity has peaked in *H. vitessoides*. In contrast, opposing patterns suggest a substantial recent increase in DNA and LTR element activity. Together with annotations of essential sesquiterpenoid hormonal pathways, neuropeptides, microRNAs and transposable elements, the high-quality genomic and transcriptomic resources we provide for the economically important moth *H. vitessoides* provide a platform for the development of genomic approaches to pest management, and contribute to addressing fundamental research questions in Lepidoptera.

38. Horseshoe crab genomes reveal the evolution of genes and microRNAs after three rounds of whole genome duplication

Hing-man Au
The Chinese University of Hong Kong

Horseshoe crab genomes reveal the evolution of genes and microRNAs after three rounds of whole genome duplication Whole genome duplication (WGD) has occurred in relatively few sexually reproducing invertebrates. Consequently, the WGD that occurred in the common ancestor of horseshoe crabs ~135 million years ago provides a rare opportunity to decipher the evolutionary consequences of a duplicated invertebrate genome. Here, we present a high-quality genome assembly for the mangrove horseshoe crab *Carcinoscorpius rotundicauda* (1.7 Gb, N50 = 90.2 Mb, with 89.8% sequences anchored to 16 pseudomolecules, 2n = 32), and a resequenced genome of the tri-spine horseshoe crab *Tachypleus tridentatus* (1.7 Gb, N50 = 109.7 Mb). Analyses of gene families, microRNAs, and synteny show that horseshoe crabs have undergone three rounds (3R) of WGD. Comparison of *C. rotundicauda* and *T. tridentatus* genomes from populations from several geographic locations further elucidates the diverse fates of both coding and noncoding genes. Together, the present study represents a cornerstone for improving our understanding of invertebrate WGD events on the evolutionary fates of genes and microRNAs, at both the individual and population level. We also provide improved genomic resources for horseshoe crabs, of applied value for breeding programs and conservation of this fascinating and unusual invertebrate lineage.

39. Genome and sex-biased transcriptomic response to temperature change of common yellow butterfly *Eurema hecabe* (Family Pieridae)

Ivy H.T. Lee[1], Wenyan Nong[1], Wai Lok So1, Yichun Xie[1], Chris K. H. Cheung[1], Ho Yin Yip1, Zhe Qu[1], Thomas Swale[2], Hon-ming Lam[3], Simon K.F. Chan[4], Alexander Hayward[5], William Bendena[6], Jerome H.L. Hui[1]
[1]School of Life Sciences, Simon F.S. Li Marine Science Laboratory, State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong; [2]Dovetail Genomics, United States of America; [3]School of Life Sciences, State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong; [4]Agriculture, Fisheries and Conservation Department, Hong Kong SAR Government; [5]University of Exeter, United Kingdom; [6]Department of Biology, Queen's University, Canada

The Lepidoptera, including butterflies and moths, is one of the most widespread insect orders in the world. They play important roles in the environment as pollinators and agricultural pests. The common yellow

butterfly *Eurema hecabe* (Family Pieridae) is a dominant species found in Hong Kong and Asia, and a high-quality genome is established in this study. The genome assembly size is 296.5 Mb with high sequence continuity (scaffold N50 = 9.3Mb) and BUSCO completeness (93.0%). Messenger RNA and small RNA transcriptomes were obtained from eight developmental stages (egg, 1st to 5th instars, pupa, adult), which allowed us to annotate a total of 20,164 protein-coding genes in the genome. Comparison to other pierid butterfly genomes revealed the reorganisation of Hox and NK homeobox genes in their last common ancestor. Adult butterflies of different sexes were cultured at three temperatures, and mRNA and microRNA transcriptomes obtained separately from head and body revealed sex-specific/biased expression, including neuropeptide and sesquiterpenoid biosynthetic pathway genes. This study established a new model to understand different environmental stresses for lepidopterans in Asia, and demonstrated the differential response of sexes under climate change.

40. Dehydration and viral infection yield similar stress that deplete glycogen and increase feeding in the Western Flower Thrips, *Frankliniella occidentalis*

Samuel T. Bailey[1], Alekhya A. Kondragunta[1], Hyojin A. Choi[1], Jinlong Han[2], Dorith Rotenberg[2], Diane E. Ullman[3], Joshua B. Benoit[1]
[1]University of Cincinnati, Department of Biological Sciences; [2]North Carolina State University, Department of Entomology and Plant Pathology; [3]University of California Davis, Department of Entomology and Nematology

Western flower thrips (*Frankliniella occidentalis*) are significant, globally invasive, generalist pests of agriculture worldwide. Additionally, thrips vector a myriad of plant-infecting tospoviruses through salivation while feeding, the most economically significant of which, Tomato Spotted Wilt Virus (TSWV), causes an annual worldwide loss of over $1 billion in crop damages. As a globally invasive pest, they are thus likely to contend with shifts in water availability across their expansive range. In this study, *Frankliniella occidentalis* were exposed to single bouts of dehydration and their phenotypic changes were analyzed through RNA-seq, nutrient reserve assays, and monitoring feeding behavior. RNA-seq analysis identified differentially expressed transcripts during dehydration and revealed, among others, a significant enrichment for GO terms related to carbohydrate transport and metabolism. A reduction of glycogen reserves was confirmed during dehydration. Behaviorally, desiccation significantly increased the feeding probability of thrips. Assessing the influence of infection with TSWV revealed a similar depletion of glycogen and increase in feeding tendency. Together, our results indicate that dehydration and infection alter glycogen reserves and influence the feeding tendencies of thrips; crucial factors that contribute to their capacity to damage crops and spread disease.

41. *Rhipicephalus microplus*_VDAC, a novel vaccine candidate, contains conserved B-cell epitopes that induce antibodies in immunized cattle

Juan Mosqueda[1], Daniel Gustavo López-Díaz[1,2], Reyna Ortega-Sánchez[1,2], Minerva Camacho-Nuez[3], Maria Martina Esperanza Perez-Soria[1,4], Rafael Jimenez-Ocampo[5], Jacqueline Elizabeth Castañeda-Ortíz[3], Diego Josimar Hernández-Silva[1], and Gabriela Aguilar-Tipacamú[6]
[1]Immunology and Vaccines Laboratory, College of Natural Sciences. Autonomous University of Queretaro; Carretera a Chichimequillas, Ejido Bolaños, Queretaro Queretaro 76140, Mexico; [2]Master's Program in Health and Sustainable Animal Production. College of Natural Sciences. Autonomous

University of Queretaro; Querétaro, Qro, Mexico; [3]Graduate program in Genomic Sciences, Autonomous University of Mexico City. San Lorenzo Núm. 290, esquina Roberto Gayol, colonia del Valle Sur, delegación Benito Juárez, México D.F., C.P. 03100; [4]Doctorado en Ciencias Biológicas. College of Natural Sciences. Autonomous University of Queretaro; Querétaro, Qro, Mexico; [5]National Institute for Forestry, Agriculture and Livestock Research—INIFAP, Experimental Field Valle del Guadiana, Durango C.P. 34170, Mexico; [6]College of Natural Sciences. Autonomous University of Queretaro; Av. de las Ciencias s/n Col Juriquilla, C.P. 76230, Queretaro, Mexico

Rhipicephalus microplus is the most widely distributed tick worldwide and causes significant economic losses in the livestock industry. It directly affects cattle by feeding on blood and damaging the skin, and indirectly acting as a vector of pathogens that cause infectious diseases, such as bovine babesiosis. Our previous studies using proteomics and bioinformatics showed that *R. microplus* VDAC (BmVDAC) is a mitochondrial porin with multiple functions and its expression is modulated by *Babesia bigemina* infection. We have demonstrated that rBmVDAC is immunogenic and that antibodies specifically recognize the native protein in the midgut of *R. microplus*. Immunization with rBmVDAC provided 82% efficacy against *R. microplus* infestation in a group of vaccinated cattle compared with a control group. These results suggest that the BmVDAC protein contains B-cell epitopes, which could be included in a vaccine for the control of tick infestation. Therefore, *R. microplus* ticks from eight different states in Mexico were collected, the BmVDAC gene was amplified, cloned, and sequenced, and by means of a multiple alignment analysis, it showed a percentage of similarity greater than 99% in all nine alleles evaluated, indicating a high degree of conservation. By bioinformatics, we identified four peptides that were predicted to contain B-cell epitopes in the amino acid sequence of BmVDAC. Each peptide was chemically synthesized, emulsified with adjuvant, and subcutaneously inoculated into two susceptible cattle four times every three weeks. Serum samples were obtained and IgG antibody responses against each peptide were assessed by indirect ELISA and analyzed by ANOVA. Only one peptide (VDAC-3), out of four evaluated, induced antibodies in both immunized cattle. The other three peptides induced antibody generation in only one of the two cattle, or induced antibodies in cattle that decreased after three immunizations. Bioinformatics analysis showed a high degree of similarity between peptides 1, 2 and 4 with *Bos taurus* VDAC. BmVDAC contains a peptide with conserved B-cell epitopes, which can be tested in a challenge against *R. microplus*. Funded by USDA-NAU (1003705-03) and FONDEC-UAQ (FNV-2020-06).

42. Evolution of non-insect/crustacean arthropods: from genomics to sesquiterpenoid endocrinology

Wai Lok So[1], Wenyan Nong[1] and Jerome Ho Lam Hu[i1]
[1]School of Life Sciences, The Chinese University of Hong Kong

The Arthropoda comprises animals that forms the largest group of extant animals in the globe. The success of arthropods has been postulated to be contributed by their unique endocrinology. Nonetheless, our understanding of these biological systems is largely coming from the studies of insects and crustaceans, and that in non-insect/crustacean arthropods remains poorly known. Thus, this project aims to expand our knowledge on the non-insect/crustacean arthropods in a genomic view of studies and explore the presence and function of the sesquiterpenoid hormonal system in non-insects/crustacean arthropods. In total, 8 myriapod genomes were sequenced and unique genetic adaptations have been revealed in genomic analyses. Besides, in silico search of the conserved hormone system genes across arthropod genomes identified a loss of juvenile hormone acid methyltransferase (JHAMT), an essential gene encodes an enzyme that synthesizes

methyl farnesoate (MF) and juvenile hormone (JH), in the millipede lineage. Subsequent hormonal treatments showed that centipede body tissues are responsive towards sesquiterpenoid hormones (farnesoic acid and MF) while millipedes do not, suggesting the system was established in the ancestor and the gene loss is a rather lineage specific event in the closely related millipedes. The newly established genomic and transcriptomic resources conducted have provided valuable sources for future investigations in relevant fields, and the biology explored here have also provided a new doorway in comprehending the evolution of arthropods, which potentially reveal how this diversified group of animals have become so successful in the history of life.

43. Metabarcoding and population genomics to assess usage of alternate habitats by *Rhyzopertha dominica* (Coleoptera: Bostrichidae)

Erin D. Scully[1], Valerie Nguyen[2], Georgina Bingham[3], Morgan Olmstead[1], and Brenda Oppert[1]
[1]USDA-ARS Center for Grain and Animal Health, Research Stored Product Insect and Engineering Research Unit, Manhattan, KS USA; [2]Kansas State University, Department of Entomology, Manhattan, KS USA; [3]University of Nebraska-Lincoln, Department of Entomology, Lincoln, NE USA

Rhyzopertha dominica (Coleoptera: Bostrichidae) is a cosmopolitan stored product pest that causes significant economic losses to raw and finished commodities worldwide. Movement and prolonged storage of infested products are major drivers of infestations in food facilities; however, these insects also commonly reside in other agricultural landscapes, including tallgrass prairies, woodlands, and evarious types of croplands. Currently, it is unclear whether insects found in these landscapes represent resident populations, migratory individuals, or spillover from populations infesting nearby food facilities. Although previous studies have documented full development of *R. dominica* reared on seeds from native grasses and acorns and prior reports have documented feeding on woody and living plant materials, it is unclear whether they persist in alternate landscapes and or whether these populations could serve as sources for infestation of nearby food facilities. To fill these knowledge gaps, we collected *R. dominica* adults in pheromone-baited Lindgren traps at three different locations in Manhattan, KS across three field seasons (2017-2019) to assess population structure and admixture at the locations over time. The locations included a commercial grain elevator that buys and sells grain from over 24 counties in Kansas, a small flour mill that primarily processes grain harvested in nearby fields (Hal Ross Flour Mill, Kansas State University), and a native tallgrass prairie (Konza Prairie Biological Research Station). We also performed DNA metabarcoding analysis using plant rbcL amplicons from insect guts collected at Konza Prairie and a second native tallgrass prairie (Nine Mile Prairie, Lincoln, NE), to assess host plant usage in these habitats. Population genomic analysis revealed extensive admixture among the three locations over time among three prominent genotypes. A likely explanation is spillover of high populations sampled at the commercial elevator to other nearby habitats. Consistent with in vitro studies, rbcL amplicons derived from native grasses, deciduous trees, and gymnosperms were also identified in both prairie populations, indicating that insects may use resources within these habitat patches as food.

FUNCTIONAL GENOMICS

44. Annotating insect regulatory genomes

Hasiba Asma[1], Ellen Tieke[2], Kevin Deem[2], Yoshinori Tomoyasu[2], and Marc S. Halfon[1,3,4]

[1]University at Buffalo; [2]Miami University; [3]NYS Center of Excellence in Bioinformatics and Life Sciences; [4]Roswell Park Comprehensive Cancer Center

Our work is focused on the rapid and inexpensive discovery and annotation of gene regulatory sequences in a wide range of sequenced insect species. Regulatory sequences-e.g. enhancers-play an essential role in controlling spatial and temporal gene expression. Variation in enhancer sequences is a driving force of speciation, making it critical to understand the mechanisms underlying enhancer evolution. Earlier attempts to study regulatory sequence evolution have been constrained in scope due the limited number of known insect enhancers outside of the well-studied *Drosophila melanogaster*. Although a growing number of insect species have had their genome sequenced (476 sequenced species), there are few known enhancers, and even fewer still from a common locus across many species. We are engaged in a systematic attempt to annotate large numbers of insect genomes, and in so doing, to collate groups of potentially homologous enhancers for further study. For this purpose, we previously developed SCRMshaw (for Supervised Cis-Regulatory Module Discovery), an effective method for computational enhancer discovery. We first create a training set of known enhancer sequences, using the REDfly database, to train SCRMshaw to guide its search for other enhancers with related functions. Due to the presence of deep enhancer homologies in distantly-related insect species, SCRMshaw, when trained on *Drosophila* sequences, can also discover enhancers in a cross-species fashion in genomes as far diverged as the 345 Ma honeybee genome, with a ~75% true-positive rate. We aim to predict enhancers in 60 or more of species with an acceptable quality of assembled genomes, and make these data publicly available in a user-friendly and accessible fashion. To date, we have successfully predicted enhancers in 26 species from orders Diptera, Hymenoptera, Coleoptera, Lepidoptera, and Hemiptera. We have also improved our analysis pipeline to accurately match genes with their Drosophila orthologs. Leveraging all of these data, we selected and experimentally validated 9 of our enhancer hits from 3 different species, using transgenic fly models. Empirical testing of these cross species SCRMshaw predictions revealed 77% (7/9) had expected regulatory activity and 100% were functional enhancers in transgenic flies. An additional 10 enhancers are currently undergoing in-vivo validation. As we assemble sets of orthologous enhancers, we expect to be able to trace their evolutionary history with unprecedented molecular details.

45. How do sizes of host-use gene families differ between specialist and generalist bark beetles?

Jared Bernard[1], Scott M. Geib[2], and Daniel Rubinoff[1]
[1]Department of Plant & Environmental Protection Sciences, University of Hawaii-Manoa, USA; [2]Pacific Basin Agricultural Research Center, Agricultural Research Service, US Department of Agriculture, USA.

What factors make some insects specialists while others can use a plethora of hosts? Of clear importance are genes that produce sensory proteins to seek out hosts as well as genes that make metabolic enzymes. Genomic studies within the last decade have pointed to larger numbers of sensory genes in generalist insects than in specialist ones. Out of necessity, these studies have compared distantly related species, which precludes insight into the mechanisms behind varying numbers of genes. By assembling and annotating the genomes of two bark beetles, the generalist *Xyleborus affinis* and the specialist *Xyleborus molokaiensis,* we increase the number of sequenced bark beetle genomes to five. As they are all within a single subfamily, we compare these genomes to learn whether sensory or metabolic gene repertoires differ with host specificity breadth, and the roles of tandem duplicates or alternative splicing. Knowledge of these mechanisms

therefore adds to our understanding of what enables pests to be successful, why rare species have restrictive habitat requirements, and how the diversity of insects has evolved.

46. Genomic analysis of the UDP-glycosyltransferase gene family in arthropods

Seung-Joon Ahn
Department of Biochemistry, Molecular Biology, Entomology & Plant Pathology, Mississippi State University, MS 39762, USA

UDP-glycosyltransferases (UGTs) are important conjugation enzymes found in all kingdoms of life, catalyzing sugar-transferring reactions with small lipophilic compounds and playing pivotal roles not only in detoxification, but in other physiological processes in insects. The glycoside conjugation increases water solubility making the compounds more easily excretable, thereby protecting the cellular system from damage by toxic compounds. It is also involved in pigment sequestration providing color to the cuticle, wings or cocoons in insects. Some UGTs are highly expressed in insect antennae, proposing a novel function in olfaction. Insect UGTs, therefore, show multifaceted roles in insect. UGTs have been identified in a number of insect genomes over the last decade and much progress has been achieved in characterizing their expression patterns and molecular functions. Since UGTs constitute one of the largest multigene families, their genomic repertoires are reflected by lineage-specific gene diversifications, along with several conserved ones within different levels of taxa. In this presentation, insect UGTs will be introduced with a recent update of the Drosophila UGTs and other arthropod UGTs from the perspectives of comparative genomics and phylogenetic analyses.

47. A historical review: Non-intact ex vivo tick culture use in tick-borne virus study and functional application

Jeffrey M. Grabowski
Foundation for Advanced Education in the Sciences at the NIH, Bethesda, MD, USA

Tick-borne viruses (TBVs) historically have caused human disease worldwide. Tick-borne flaviviruses (TBFVs) are found across Eurasia and in North America. Some TBFVs are known to be highly pathogenic, while some TBFVs of low neurovirulence have been used to model pathogenic TBFV infection, in general. There is limited knowledge on molecular interactions of viruses with ticks. Non-intact tick culture development over the decades has aided research to identify tick cells and/or tick genes functionally involved in TBFV infection. In this brief review, a focus on published literature from the 1960s until 2021 will highlight how non-intact ex vivo tick culture development has aided the study of TBV infection. This includes content on different non-intact ex vivo tick cultures, virological and molecular/immunological techniques to determine infection in these types of cultures, and how functional genomics were adapted in these setups to confirm a proviral tick host factor in salivary gland (the last barrier to transmission) cultures.

48. Functional characterization of sex-specific yeast interfering RNA larvicides facilitates mosquito sex separation and provides insight into the evolution of dipteran insect sex chromosomes and dosage compensation

Teresia Njoroge, Keshava Mysore, and Molly Duman Scheel

Indiana University School of Medicine, South Bend, IN, United States and Eck Institute for Global Health, University of Notre Dame, IN, United States

The discovery of sex-specific loci in dipteran species has uncovered genes that are required for sex-specific development. Our RNAi screens in *Aedes aegypti* larvae have uncovered multiple genes adjacent to the M/m sex determination locus that are required for survival of female larvae or the development of female-specific traits. These include many long non-coding RNAs, for which orthologs have not yet been identified in other dipteran insects, as well as protein-encoding genes that are well-conserved among arthropods. RNAi-mediated silencing of several of the protein-encoding genes in *A. aegypti* as well as other species of mosquitoes, including *Aedes albopictus*, *Anopheles gambiae*, *Culex pipiens*, and *Culex quinquefasciatus*, significantly increased adult male:female ratios. Larval consumption of *Saccharomyces cerevisiae* (yeast) strains engineered to express interfering RNA corresponding to these genes results in significant female death, increasing male:female ratios without impacting male survival or fitness. These female-specific RNAi-based yeast larvicides could benefit the implementation of emerging mosquito population control strategies that depend on mass rearing and release of adult male mosquitoes. To this end, current efforts are focusing on scaled production of the yeast larvicides and incorporation of the yeast into larval diets used in mosquito mass-rearing facilities. Characterization of these larvicides is also providing novel insights into the study of sex-specific developmental genetics, as well as the evolution of sex chromosomes and dosage compensation in dipteran insects.

49. Phenotypic and transcriptomic analyses of abnormal male sexual development in *Aedes aegypti* and *Aedes mascarensis* backcross progeny

Jiangtao Liang[1], Lin Kang[2], Pawel Michalak[2], and Igor V. Sharakhov[1]
[1]Department of Entomology, Virginia Tech, Blacksburg, VA 24061, USA [2]Edward Via College of Osteopathic Medicine, Monroe, LA 71203, USA

Manipulation of the sex determination pathway can be used for efficient sex separation and effective reduction of target mosquito populations. When male hybrids of *Aedes aegypti* females and *Aedes mascarensis* males are backcrossed to *Ae. aegypti* females, one part of the backcross progeny consists of sterile intersexes. In this study, we characterized developmental abnormalities associated with intersex phenotypes in the backcrosses and analyzed gene expression in intersexes. To test the genetic sex of intersex individuals, we conducted PCR using primers for the gene Nix, which is a male-determining factor in *Ae. aegypti*. Despite genetically being males, as shown by the presence of the Nix gene, the intersex individuals had feminized antennae, external genitalia, and reproductive organs consisting of both ovarian and testicular parts. We conducted RT-PCR using splice variant specific primers and RNA-seq to compare gene expression between intersex and normal individuals. The male-determining gene Nix expressed in the intersexes as in normal males. However, the downstream genes doublesex and fruitless expressed both male and female splice variants in the intersex individuals. The transcriptomic analysis revealed that most of the female-specific genes are expressed in intersex carcasses and reproductive organs at levels similar to that in normal females. Many male-specific genes are down-regulated in intersex carcasses and reproductive organs in comparison with normal males. These results suggest that the intersex phenotypes are caused by a malfunctional interaction between the *Ae. mascarensis* gene(s) in the M locus and the *Ae. aegypti* downstream targets, possibly repressors of female development, of the sex determination pathway. We also speculate that the evolution of the sex determination pathway in *Aedes* can potentially contribute to the diversification of mosquito species.

50. Comparisons of human and non-human feeding *Anopheles* mosquitoes link olfactory genes to anthropophily

Luke Ambrose[1], Iva Popvic[1], James Hereward[1], Daniel Ortiz-Barrientos1 and Nigel W. Beebe[1,2]
[1]School of Biological Sciences, The University of Queensland, Brisbane, Australia; [2]CSIRO, Brisbane, Queensland, Australia

The genetic basis of anthropophily (the use of humans as hosts) in mosquitoes has been studied for over a decade. While significant progress has been made in this area, research to date has focused primarily on two study systems (the global species *Aedes aegypti* and the African *Anopheles gambiae* complex). We introduce a relatively unstudied system, the *Anopheles farauti* complex from the southwest Pacific, as ideal for investigating the functional genomics of anthropophily in mosquitoes. This species complex has repeatedly colonized an archipelago in the Pacific, and in doing so, has repeatedly lost the ability to feed on humans. By sequencing the genomes of closely related lineages in the complex, isolating >200 olfactory genes and performing tests of selection on these genes, we investigate how selection may have acted on olfactory genes in this complex during host shifts associated with island colonization. Additionally, we identify and analyze evolutionary patterns in these genes which may signify a role in anthropophily. Overall, we find that most olfactory genes have been under purifying selection, with evidence of positive or relaxed selection on some genes. Based on evolutionary patterns (phylogenetic relationships, fixed amino acid differences, structural differences and kA/kS) as well as results from selection analyses, we identify numerous genes that are likely to play an important role in mosquitoes' ability to detect humans as hosts. These findings provide directions for future research into the function of these genes in mosquito anthropophily and have important implications for vector and disease control efforts.

51. CRISPR-Cas9 mutagenesis creates an *Aedes aegypti* mutant lacking light-evoked behavioral responses

Alexis M. Waldschmidt[1], **Cora E. Anderson**[1], Matthew R. Gregory[1], Brian Dineen[1], Alex M. Dittmar[1], Corey S. Nishimura[1], Jeremy T. Sutterer[1], Hannah Cunniff[1], Claire A. Lohman[1], Calla M. Sullivan[1], Elaine Teeters[1], Michelle A. Whaley[1], and Joseph E. O'Tousa[1]
[1]Department of Biological Sciences, University of Notre Dame, USA

Mosquito host detection and other disease vectoring behaviors are prompted by multiple sensory inputs, notably olfaction, vision, and heat detection. The *Aedes aegypti* β-class phospholipase C (PLCβ) is the ortholog of *Drosophila* NORPA. In *Drosophila*, NORPA function is essential for light detection in both larvae and adults. To study the role of visual inputs in *Aedes* behaviors, we created CRISPR-CAS9 induced-mutations within the *Aedes aegypti* PLCβ gene, hereafter referred to as the *norpA* gene. We present the characterization of norpA1, a homozygous viable *Aedes norpA* mutant. Molecular analysis shows that norpA1 contains a nonsense point mutation and two small deletions within the CRISPR-targeted region. An antibody designed to recognize the 124 kD PLCβ protein readily detects a protein of this size in wild type head extracts but not in norpA1 head extracts. In histological analyses, this protein was detected in the rhabdomeres of wild type photoreceptor cells but was not detected in norpA1 retinal tissues. Despite the inability to detect the PLCβ protein in norpA1, electroretinographic analyses revealed a small response to

light stimuli. This response showed slow kinetics to light on and light off relative to the wild type. To determine if the altered physiology results in the norpA1 mutant having impaired behavioral responses, we created assays to measure the light-avoidance activity of larvae and the light-triggered startle responses of both larvae and adults. In these behavioral tests, wild type mosquitoes responded to visual input while the norpA1 mutant did not. The norpA1 mutant showed normal locomotion and circadian activity, demonstrating that their lack of immediate responses to light is not due to general lethargy. These results support the conclusion that the norpA1 mutant is visually impaired, thus this mutant is a valuable genetic model that can be used to elucidate the role of vision in complex mosquito behaviors.

52. Genome-wide profiling of Kruppel Homolog 1 (Kr-h1) in *Aedes aegypti* females throughout egg maturation using CUT&RUN

Katara Griffith
Genetics, Bioinformatics, and Computational Biology, Virginia Polytechnic Institute and State University

Aedes aegypti mosquitoes are a major vector for transmitting infectious diseases such as yellow fever, dengue, chikungunya, and Zika. Male mosquitoes do not bite, while female mosquitoes are responsible for disease transmission. A better understanding of mosquito reproduction will reveal effective targets for transmission control. Kruppel homolog 1 (Kr-h1), a gene that is regulated by both 20-hydroxyecdysone (20E) and juvenile hormone, is active throughout post-embryonic development. Kr-h1 is also required for proper expression of 20E-responsive genes in female mosquitoes during egg production, following blood feeding. This transcription factor has previously been identified as either an activator or repressor for the expression of various hormone-responsive genes, though its molecular mechanism remains largely unknown. Here we use the Cleavage Under Target and Release Using Nuclease (CUT&RUN) technique to examine genome-wide Kr-h1 binding profiles throughout the egg maturation process in adult female mosquitoes. The results not only verified *Kr-h1* binding sites previously identified by chromatin immunoprecipitation assays but also open the door to defining the role of Kr-h1 in modulating chromatin accessibility of its target genes in female mosquitoes.

53. Cytochrome P450s in green peach aphid facilitate its transgenerational tolerance to indole glucosinolate-mediated plant defense

Rui Ji, Jiaxin Lei and **Keyan Zhu-Salzman**
Department of Entomology, Texas A&M University, College Station, TX, USA

Promoting indole glucosinolate production in plants increases host defense against aphid infestation. Little is known of how aphids respond to this group of plant defense compounds or of the underlying molecular mechanism. Arabidopsis thaliana CCA1-ox line over-produces indole glucosinolates. Aphids reared on CCA1-ox for over 40 generations (namely the CCA population) became less susceptible to CCA1-ox than aphids maintained on the wild-type Col-0 (namely the COL population). This elevated tolerance remained for at least eight generations after the CCA population was transferred to Col-0. Transcriptome analysis indicated that all differential cytochrome P450 monooxygenase genes (MpCYPs) were more highly expressed in the CCA population. Application of a P450 inhibitor to the CCA population resulted in decreased aphid reproduction on CCA1-ox, which was not observed if aphids were reared on Col-0. When indole glucosinolate biosynthesis in CCA1-ox was blocked using virus-induced gene silencing, the effect of the P450 inhibitor on the CCA population was attenuated, affirming the essential role played by MpCYPs in

counteracting the defense mechanism in CCA1-ox that is low or absent in Col-0. Furthermore, we used host-induced gene silencing to identify MpCYP380C6 and MpCYP380C9 that specifically facilitated the CCA population to cope with CCA1-mediated plant defense. Expression profiles revealed their possible contribution to the transgenerational tolerance observed in aphids.

MICROBIOME

54. Overabundance of *Asaia* and *Serratia* bacteria is associated with deltamethrin insecticide susceptibility in *Anopheles coluzzii* from Agboville, Côte d'Ivoire

Bethanie Pelloquin[1,2], Mojca Kristan[1], Constant Edi[3], Anne Meiwald[1], Emma Clark[1], Claire L. Jeffries[1], Thomas Walker[1], Nsa Dada[4,5,6], and Louisa A. Messenger[1]
[1]Department of Disease Control, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; [2]School of Tropical Medicine and Global Health, University of Nagasaki, Nagasaki, Japan; [3]Centre Suisse de Recherche Scientifique en Côte d'Ivoire, Abidjan, Côte d'Ivoire; [4]Faculty of Science and Technology, Norwegian University of Life Sciences, Aas, Norway; [5]Public Health and Epidemiology Department, Nigerian Institute of Medical Research, Lagos, Nigeria [6]Tropical Infectious Disease Research Center, University of Abomey-Calavi, Cotonou, Benin

Insecticide resistance among mosquito species is now a pervasive phenomenon that threatens to jeopardize global malaria vector control efforts. Evidence of links between the mosquito microbiota and insecticide resistance is emerging, with significant enrichment of insecticide degrading bacteria and enzymes in resistant populations. Using 16S rRNA amplicon sequencing, we characterized and compared the microbiota of *Anopheles coluzzii* in relation to their deltamethrin resistance and exposure profiles. Comparisons between 2- and 3-day-old deltamethrin-resistant and -susceptible mosquitoes demonstrated significant differences in microbiota diversity. *Ochrobactrum*, *Lysinibacillus*, and *Stenotrophomonas* genera, each of which comprised insecticide-degrading species, were significantly enriched in resistant mosquitoes. Susceptible mosquitoes had a significant reduction in alpha diversity compared to resistant individuals, with *Asaia* and *Serratia* dominating microbial profiles. There was no significant difference in deltamethrin-exposed and -unexposed 5- to 6-day-old individuals, suggesting that insecticide exposure had minimal impact on microbial composition. *Serratia* and *Asaia* were also dominant in 5- to 6-day-old mosquitoes, which had reduced microbial diversity compared to 2- to 3-day-old mosquitoes. Our findings revealed significant alterations of *A. coluzzii* microbiota associated with deltamethrin resistance, highlighting the potential for identification of novel microbial markers for insecticide resistance surveillance. qPCR detection of *Serratia* and *Asaia* was consistent with 16S rRNA sequencing, suggesting that population-level field screening of bacterial microbiota may be feasibly integrated into wider resistance monitoring, if reliable and reproducible markers associated with phenotype can be identified.

55. Chromosomal-level assembly of *Bactericera cockerelli* reveals rampant gene family expansions and duplications of horizontally transferred genes that impact insect-microbe-plant-interactions

Younghwan Kwak[1], Jacob A. Argandona[1], Patrick H. Degnan[2*], and **Allison K. Hansen**[1*]

[1]Department of Entomology, University of California, Riverside, 900 University Ave Riverside, CA 92521 USA [2]Department of Microbiology and Plant Pathology, University of California, Riverside, 900 University Ave Riverside, CA 92521 USA

Lineage specific expansions and gene duplications are some of the most important sources of evolutionary novelty in eukaryotes. Although not as prevalent in eukaryotes compared to bacteria, horizontal gene transfer events can also result in key adaptations for insects, especially for those involved in insect-microbe interactions. In this study we assemble the first chromosomal assembly of the psyllid *Bactericera cockerelli* and reveal that the *B. cockerelli* genome has experienced significantly more gene expansion events compared to other Hemipteran representatives with fully sequenced genomes. We also reveal that *B. cockerellia's* genome is the largest psyllid genome (567 Mb) sequenced to date and is ~15% larger than the other two psyllid species genomes sequenced (*Pachypsylla venusta* and *Diaphorina citri*). Structurally, *B. cockerelli* appears to have an additional chromosome compared to the distantly related psyllid species *P. venusta* due to a previous chromosomal fission or fusion event. The increase in genome size and dynamic nature of the *B. cockerelli* genome may largely be contributed to the widespread expansion of type I and type II repeat elements that are rampant across all of *B. cockerellia's* chromosomes. These repeat elements are distributed near equally in both euchromatic and heterochromatic regions. Furthermore, significant gene family expansions and gene duplications were uncovered for genes that are expected to be important in its adaptation to insect-plant and microbe interactions, which include transcription factors, proteases, odorant receptors, and horizontally transferred genes that are involved in the nutritional symbioses with their long-term nutritional endosymbiont *Carsonella*.

56. Hidden structural diversity within a *Wolbachia* strain infecting cherry-infesting *Rhagoletis* (Diptera: Tephritidae) flies across North America

Daniel J. Bruzzese[1], Thomas M. Wolfe[2], Hannes Schuler[3,4], Wee L. Yee[5], Juan Rull[6,7], Martin Aluja[6], Robert B. Goughnour[8], Glen Ray Hood[9], Christian Stauffer[2], and Jeffery L. Feder[1]
[1]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN,USA; [2]Department of Forest and Soil Sciences, Boku, University of Natural Resources and Life Sciences, Vienna, Austria; [3]Faculty of Science and Technology, Free University of Bozen-Bolzano, Bozen-Bolzano, Italy; [4]Competence Centre for Plant Health, Free University of Bozen-Bolzano, Bozen-Bolzano, Italy; [5] United States Department of Agriculture, Temperate Tree Fruit & Vegetable Research Unit, Agricultural Research Service, Wapato, WA, USA; [6]Instituto de EcologÃa A.C., Xalapa, México; [7] LIEMEN-División Control Biológico de Plagas, PROIMI Biotecnología-CONICET, Tucumán, Argentina; [8]Washington State University Extension, Vancouver, WA, USA; [9]Department of Biological Sciences, Wayne State University, Detroit, MI, USA

The maternally inherited endosymbiont *Wolbachia* infects over 50% of all insects and can have great effects on host fitness ranging from mutualism to reproductive parasitism. Successfully introduced into *Aedes* populations to control spread of Dengue, *Wolbachia* are becoming a key tool for vector control. *Wolbachia* genomes are rich in transposable elements, phages, and other repetitive elements, all of which can be transferred between strains coinfecting a single host via horizontal gene transfer (HGT). Taken together, *Wolbachia* genomes are incredibly dynamic with the potential for rapid change. Whole genome sequencing has recently identified hidden genetic diversity within single Wolbachia strain that was previously overlooked by traditional molecular barcoding techniques that used only a few loci. However, genome structure diversity within a *Wolbachia* strain remains mostly unknown. Here, we investigate the structural

diversity and gene content of a *Wolbachia* strain, wCin2, that infects cherry-infesting *Rhagoletis* fruit fly species (Diptera: Tephritidae) across North America and in some host populations coinfects with other Wolbachia strains. We develop a high-yield, high molecular weight DNA extraction technique for *Rhagoletis* that allows us to prepare a sequencing library from a single fly. We demonstrate that from a single host fly it is now possible to use Oxford Nanopore sequencing to assemble closed *Wolbachia* genomes even if multiple Wolbachia strains are present. With our assemblies, we then compare *Wolbachia* genome structure and gene content between geographically distinct cherry-infesting *Rhagoletis* populations and confirm possible HGT events between coinfecting *Wolbachia* strains. Our results will help better understand trajectories of *Wolbachia* infections in natural populations and will be important to assess the risks for structural changes and the acquisition of novelties in released *Wolbachia* strains that may complicate using Wolbachia as a biocontrol element.

57. Delivery of a genetically marked Serratia AS1 to medically important arthropods for use in RNAi and paratransgenic control strategies.

Mona Koosha[1], Hassan Vatandoost[1], Fateh Karimian[2], Nayyereh Choubdar[1], and **Mohammad Ali Oshaghi[1]**
[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran [2]Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran

Understanding how arthropod vectors acquire their bacteria is essential for implementation of paratransgenic and RNAi strategies using genetically modified bacteria to control vector-borne diseases. In this study, a genetically marked Serratia AS1 strain expressing the mCherry fluorescent protein (mCherry-Serratia) was used to test various acquisition routes in six arthropod vectors including *Anopheles stephensi*, *Culex pipiens*, *Cx. quinquefaciatus*, *Cx. theileri*, *Phlebotomus papatasi*, and *Hyalomma dromedarii*. Depending on the species, the bacteria were delivered to (i) mosquito larval breeding water, (ii) host skin, (iii) sugar bait, and (iv) males (paratransgenic). The arthropods were screened for the bacteria in their guts or other tissues. All the hematophagous arthropods were able to take the bacteria from the skin of their hosts while taking blood meal. The mosquitoes were able to take up the bacteria from the water at larval stages and to transfer them transstadially to adults and finally to transfer them to the water they laid eggs in. The mosquitoes were also able to acquire the bacteria from male sperm. The level of bacterial acquisition was influenced by blood feeding time and strategies (pool or vessel feeding), dipping in water and resting time of newly emerged adult mosquitoes, and the disseminated tissue/organ. Transstadial, vertical, and venereal bacterial acquisition would increase the sustainability of the modified bacteria in vector populations and decrease the need for supplementary release experiments whereas release of paratransgenic males that do not bite has fewer ethical issues. Furthermore, this study is required to determine if the modified bacteria can be introduced to arthropods in the same routes in nature.

58. Gut microbiota of sand fly vectors of zoonotic visceral Leishmaniasis (ZVL); Host-environment interplay shapes diversity

Fateh Karimian[1], Nayyereh Choubdar[2], Mona Koosha[2], and **Mohammad Ali Oshaghi[2*]**
[1]Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran [2]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

The development of *Leishmania* parasites within sand fly vectors occurs entirely in the insect gut lumen, in the presence of symbiotic and commensal bacteria. The impacts of host species and environment on the gut microbiome are currently poorly understood. We employed MiSeq sequencing of the V3-16S rRNA gene amplicons to characterize and compare the gut microbiota of field-collected populations of *Phlebotomus kandelakii*, *P. perfiliewi*, *P. alexandri*, and *P. major*, the primary or secondary vectors of zoonotic visceral leishmaniasis (ZVL) in three distinct regions of Iran where ZVL is endemic. In total, 160,550 quality-filtered reads of the V3 region yielded a total of 72 operational taxonomic units (OTUs), belonging to 23 phyla, 47 classes, 91 orders, 131 families, and 335 genera. More than 50% of the bacteria identified were Proteobacteria, followed by Firmicutes (22%), Deinococcus-Thermus (9%), Actinobacteria (6%), and Bacteroidetes (5%). The core microbiome was dominated by eight genera: *Acinetobacter*, *Streptococcus*, *Enterococcus*, *Staphylococcus*, *Bacillus*, *Propionibacterium*, *Kocuria*, and *Corynebacterium*. *Wolbachia* were found in *P. alexandri* and *P. perfiliewi*, while Asaia sp. was reported in *P. perfiliewi*. Substantial variations in the gut bacterial composition were found between geographically distinct populations of the same sand fly species, as well as between different species at the same location, suggesting that sand fly gut microbiota is shaped by both the host species and geographical location. *Phlebotomus kandelakii* and *P. perfiliewi* in the northwest, and *P. alexandri* in the south, the major ZVL vectors, harbor the highest bacterial diversity, suggesting a possible relationship between microbiome diversity and the capacity for parasite transmission. In addition, large numbers of gram-positive human or animal pathogens were found, suggesting that sand fly vectors of ZVL could pose a potential additional threat to livestock and humans in the region studied. The presence of *Bacillus subtilis*, *Enterobacter cloacae*, and *Asaia* sp suggests that these bacteria could be promising candidates for a paratransgenesis approach to the fight against Leishmaniasis.

59. Microbiome analysis of the New World Screwworm from wild populations, mass-rearing colonies, and transgenic strain

Alex P. Arp
USDA-ARS

Domestication of wild strains for laboratory research or biological control programs can have a multitude of effects on the insect physiology, behavior, and genetics due to selective pressures from captive rearing environments. These changes can have significant impact on research observations and possibly reduce insect fitness in the wild when released for biological control, such as the sterile insect technique. The microbiome of captive insects is often greatly reduced in laboratory colonies due to disruptions in the natural vertical or horizontal transfer, such as bleaching embryos, rearing on sterile diet media, or from antibiotics used to suppress transgenic expression systems. Here we report differences in *Cochliomyia hominivorax* microbiomes from naturally occurring myiases, mass reared colonies, and transgenic Tet-off strains. Significant differences were observed between all three groups, with wild captured flies having the most divergent and diverse microbial composition. Interestingly, the transgenic flies created from production strains and reared on a combination of potassium permanganate and tetracycline did not have lower microbial diversity than the production strain. Additionally, *Proteus sp*. which are associated with blowfly oviposition stimulant volatiles, were in low abundance across treatments. In wild samples, which had differing microbiota among myiases, adult flies had many manure-related bacteria and potentially

infectious bacteria of veterinary importance. Overall, this study has provided the screwworm eradication program a platform to continue exploring the effects associated bacteria have on screwworm fitness.

60. Microbial symbiont curation through innate immune system evolution

Kyle Buffin[1] and Dr. Zakee Sabree[1]
[1]Department of Evolution, Ecology, and Organismal Biology, The Ohio State University

The complexity of host and microbial symbioses varies drastically across arthropods; however, over evolutionary time scales the governing factors of microbial community assembly and symbiotic interactions are not well understood. Host lifestyle and diet certainly contribute to microbial exposure and nutrient availability, but community assembly is also influenced by host factors including the innate immune system which acts as an interface between host and symbiont. The function of this detection and selection process is essential, as the microbiome is consistently shown to be implicated in host development and health through its role in important enzymatic processes. To this end we hypothesize that the evolutionary history of host immune genes correlates with microbiome composition shifts across arthropod species. Previous research has identified that innate immune gene families show differing evolutionary dynamics. While some species of arthropods have lost entire innate immune pathways, it is just as common others have gained novel gene functions through duplication and diversification. As host immune genes have duplicated and diverged over the course of evolution, different arthropod species have essentially developed a specific ecosystem in which to harbor its microbiome. To evaluate this concept, we propose a combination of comparative genomics of innate immune genes from host species spanning the Arthropoda phylum, in addition to analysis of microbiome community composition. A hypothesized outcome from this research is an observable correlation between a host's number of innate immune genes and their symbiotic microbial species diversity. We also will interrogate how specific evolutionary characteristics, such as diversification of recognition proteins, has impacted microbiome assembly.

61. Evolution of endosymbiont protein-complexes through label-free proteomics

Gerald Maeda[1], Vy Dang[2], Mary Kate Kelly[1], Edward Marcotte[2], Nancy Moran[1]
[1]Department of Integrative Biology University Texas, Austin; [2]Department of Molecular Biosciences University Texas, Austin

To-date, the smallest genomes of cellular organisms are found within bacterial endosymbionts associated with sap-feeding insects. Genome shrinkage and gene loss are hallmarks of long-term host association and have resulted in endosymbiont genomes comparable to those of large organelles. The loss of coding capacity for proteins that participate in larger protein complexes begs the question of how these complexes assemble, what binding partners may have changed, and which remaining complexes are truly stable in vivo. The relationship between the pea aphid, *Acyrthosiphon pisum*, and its obligate endosymbiont, *Buchnera aphidicola*, is one of the best-studied obligate symbioses, and offers an opportunity to study how extensive genome reduction has led to changes in the composition of known protein complexes. Using the aphid-*Buchnera* system, we are applying several label-free, proteomics-based techniques to catalog changes in

Buchnera protein complexes and to identify host-symbiont protein-protein interactions. Together, these approaches will be useful in understanding the evolution of protein complexes within endosymbionts that have experienced extreme genome reduction, and also for identifying novel, protein-mediated interactions between host and symbiont.

62. Symbiont-mediated gene knockdown of honey bees (*Apis mellifera*)

Jo-anne Holley[1], P.J. Lariviere[2], Dennis Mishler[2], Nancy Moran[1], Jeffrey Barrick[2]
[1]Department of Integrative Biology, University of Texas at Austin, USA; [2]Molecular Biosciences, University of Texas at Austin, USA. The

Western honey bee, *Apis mellifera*, is a globally distributed and domesticated pollinator in agroecosystems as well as a model organism for understanding social behavior. Yet, despite its importance in agriculture and scientific research, there is a shortage of genetic tools, due in part to the complex social life cycle in which only the queen reproduces. RNA interference (RNAi) can be used for knocking down honey bee gene expression, but traditional delivery modalities such as dsRNA feeding and injection can be laborious, costly, and injurious to the bee. Here we report on the development, optimization, and dissemination of a system for symbiont-mediated RNAi in honey bees, which aims to overcome these drawbacks. In this system, a naturally occurring honey bee bacterial gut symbiont, *Snodgrassella alvi*, is engineered to express a dsRNA construct targeting the bee gene of interest. Bees are then inoculated with engineered *S. alvi* and screened for the phenotype of interest, and transcript levels are quantified with qPCR to validate gene knockdown. Prior work demonstrated the feasibility of this approach through successful knockdown of insulin receptor and defensin genes. We are currently studying genes predicted to be involved in three processes: basic cell homeostasis (i.e., essential gene function), locomotion, and feeding/metabolism. Adult bees inoculated with *S. alvi* containing these knockdown constructs will be screened for emergent phenotypes: increase in mortality rate, paralysis and/or a decrease in tracked movement, and change in body weight and/or sucrose responsiveness. In parallel, we are actively engaging in scientific outreach to ensure this toolkit is accessible to the honey bee research community. First, undergraduates from two lab-based year-long courses within the UT Freshman Research Initiative are building dsRNA constructs and conducting phenotype assays to demonstrate the toolkit's feasibility. Second, we are seeking collaborations with honey bee researchers to propose potential target genes or to receive training and materials to utilize this toolkit on their own.

63. An integrated overview of the bacterial flora composition of *Hyalomma anatolicum,* the main vector of CCHF

Nayyereh Choubdar[1], Fateh Karimian[2], Mona Koosha[1], and **Mohammad Ali Oshaghi**[1]
[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [2]Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran

The microbial flora associated with *Hyalomma anatolicum* ticks was investigated using culture-dependent (CD) and independent (next generation sequencing, NGS) methods. The bacterial profiles of different organs, development stages, sexes, and of host cattle skins were analyzed using the CD method. The egg and female gut microbiota were investigated using NGS. Fourteen distinct bacterial strains were identified

using the CD method, of which *Bacillus subtilis* predominated in eggs, larval guts and in adult female and male guts, suggesting probable transovarial transmission. *Bacillus velezensis* and *B. subtilis* were identified in cattle skin and tick samples, suggesting that skin is the origin of tick bacteria. *H. anatolicum* males harbour lower bacterial diversity and composition than females. The NGS analysis revealed five different bacterial phyla across all samples, *Proteobacteria* contributing to >95% of the bacteria. In all, 56611sequences were generated representing 6,023 OTUs per female gut and 421 OTUs per egg. Francisellaceae family and *Francisella* make up the vast majority of the OTUs. Our findings are consistent with interference between *Francisella* and *Rickettsia*. The CD method identified bacteria, such *B. subtilis* that are candidates for vector control intervention approaches such paratransgenesis whereas NGS revealed high *Francisella* spp. prevalence, indicating that integrated methods are more accurate to characterize microbial community and diversity.

List of Participants:

Name	Institution	Email Address
A G, Triveni	M S Ramaiah College Of Arts Science And Commerce	triveni.ag@gmail.com
Abo-Shnaf, Reham	Agricultural Research Centre	riamaboshnaf@yahoo.com
Abreu Reis, Manoely	UFRPE	manoelyabreu@gmail.com
Abreu Silva, Fernando Allan	UNIFESP (Universidade Federal de São Paulo)	abreusilvaf@gmail.com
Acford-Palmer, Holly	London School of Hygiene & Tropical Medicine	Holly.Acford-Palmer1@student.lshtm.ac.uk
Afrane, Yaw	University of Ghana	yafrane@ug.edu.gh
Aguado, Lauren	Rockefeller University	laguado@rockefeller.edu
Ahmed, Ayman	Institute of endemic disease, University of Khartoum	ayman.ame.ahmed@gmail.com
Ahmed-Braimah, Yasir	Syracuse University	yahmed@syr.edu
Ahn, Seung-Joon	Mississippi State University	seungjoon.ahn@msstate.edu
Akoth, Modesta	Egerton University	modestakoth4@gmail.com
Alvares-Jarreta, Jorge	EMBL-EBI	jalvarez@ebi.ac.uk
Ambrose, Luke	The University of Queensland	l.ambrose@uq.edu.au
Amvongo-Adjia, Nathalie	Centre for Research in Infectious Diseases (CRID)	nathalie.amvongo@crid-cam.net
Anderson, Cora	University of Notre Dame	cander34@nd.edu
Andrade, Tamires	Vale Institute of Technology	tamiresdeoliveirandrade@gmail.com
Andrysiak, Nick	University of Notre Dame	nandrysi@nd.edu
Antonino, José Dijair	Universidade Federal Rural de Pernambuco	dijair.antonino@ufrpe.br
Anyieni, Ruth	Jomo kenyatta university of agriculture and technology	ruthanyieni15@gmail.com
Araujo Hamasaki, Lucas Takashi	Universidade Federal de Alfenas	lucas.hamasaki@sou.unifal-mg.edu.br
Araújo, Maisa	Fiocruz Rondônia	maisa.araujo@fiocruz.br
Arbetman, Marina	Comahue University & CONICET	marbetman@comahue-conicet.gob.ar
Arensburger, Peter	Cal Poly Pomona	parensburger@cpp.edu
Arp, Alex	USDA-ARS	alex.arp@usda.gov
Asma, Hasiba	State University at Buffalo	hasibaas@buffalo.edu
Au, Hing-man	CUHK	candyr2117@gmail.com
Awuoche, Erick	Yale University	erick.awuoche@yale.edu

Ayaz, Mazhar	Cholistan University of Veterinary And Animal Sciences,	mmazharayaz@cuvas.edu.pk
Azeredo-Espin, Ana	Campinas State University (Unicamp)	amlazeredo@gmail.com
Babatunde, Olugbenga	Federal University of Technology, Akure	osbabatunde@futa.edu.ng
Bailey, Sam	University of Cincinnati	bailest@mail.uc.edu
Barillas-Mury, Carolina	National Institutes of Health, USA	cbarillas@niaid.nih.gov
Bedasso, Alemnesh	ETHIOPIA PUBLIC HEALTH INSTITU	entomologyat@yahoo.com
Benoit, Joshua	University of Cincinnati	joshua.benoit@uc.edu
Bernard, Jared	University of Hawaii–Manoa	bernardj@hawaii.edu
Bhattarai, Upendra	Harvard University	upendrabhattarai@fas.harvard.edu
Bickerstaff, James	CSIRO	james.bickerstaff@csiro.au
Blissard, Gary	Boyce Thompson Institute	gwb1@cornell.edu
Bon, Marie-Claude	USDA-ARS-EBCL	mcbon@ars-ebcl.org
Brooks, Elizabeth	University of Notre Dame	ebrooks5@nd.edu
Brown, Katy	University of Cambridge	kab84@cam.ac.uk
Bruzzese, Daniel	University of Notre Dame	dbruzzes@nd.edu
Buchon, Nicolas	Cornell University	nicolas.buchon@cornell.edu
Buffin, Kyle	The Ohio State University	buffin.2@buckeyemail.osu.edu
Burke, Gaelen	University of Georgia	grburke@uga.edu
C dos Santos, Najara A	Fiocruz Rondônia	najara.akira@fiocruz.br
Cabral-de-Mello, Diogo	UNESP	cabral.mello@unesp.br
Campino, Susana	London School of Hygiene & Tropical Medicine	susana.campino@lshtm.ac.uk
Canova, Alyssa	Texas A&M University	acanova@tamu.edu
Castillo, Julio	INDICASAT AIP	juliocastillo25@gmail.com
Catto, Michael	University of Georgia	catto.michael@gmail.com
Chabi, Joseph	VectorLink	joseph_chabi@pmivectorlink.com
Chakraborty, Souvik	University of Cincinnati	chakras7@mail.uc.edu
Charamis, Jason	IMBB, Foundation for Research and Technology	jason_charamis@imbb.forth.gr
Chen, Mengyao	Zhejiang University	mengyaochen@zju.edu.cn
Chen, Yolanda	University of Vermont	yolanda.chen@uvm.edu
Childers, Anna	USDA-ARS	anna.childers@usda.gov
Choubdar, Nayyereh	Tehran University Of Medical Sciences	nchoubdar@yahoo.com
Christensen, Blair	University of Vermont	blair.christensen@uvm.edu

Christophe, Antonio Nkondjio	OCEAC	antonio_nk@yahoo.fr
Coates, Brad	USDA ARS	brad.coates@usda.gov
Coggins, Bret	University of Notre Dame	bcoggins@nd.edu
Contreras, Winnie	Universidad Peruana Cayetano Heredia	winnie.contrerar@upch.pe
Costa, Gabriel	Universidade Federal de São Paulo	gabrielgnr@gmail.com
Cunha, Vanessa	University of Sao Paulo	vanscunha@gmail.com
Dada, Nsa	Tropical Infectious Diseases Research Centre	nsadada@yahoo.com
Dalikova, Martina	University of Kansas	m.dalikova@gmail.com
Dare, Christianah Adebimpe	Osun State University Osogbo Nigeria	bimpeoasis@yahoo.com
Dave, Kirtan	GSFC University	kirtandave7@gmail.com
de Oliveira, Lucas	IRSC	deoliveiralm@mail.irsc.edu
Debnath, Rajal	SERIBIOTECH RESEARCH LABORATORY	rajal.debnath@gmail.com
Debrah, Isaiah	West Africa Centre for Cell Biology of Infectious Pathogen, University of Ghana	isaiah20009@gmail.com
D'Elia, Tom	Indian River State College	tdelia@irsc.edu
Devilliers, Julien	University of Leicester	jsad1@leicester.ac.uk
Diaz, Alejandro	Institute of sciences, BUAP	alexhalos61@gmail.com
Dietrich, Isabelle	The Pirbright Institute	Isabelle.Dietrich@pirbright.ac.uk
Domann, Sara	Kansas State University	saradomann@ksu.edu
Dowle, Eddy	University of Otago	eddy.dowle@otago.ac.nz
Dubey, Himanshu	Seri-Biotech Research Laboratory, Bangalore	hemu.bt@gmail.com
Duman Scheel, Molly	Indiana University School of Medicine	mscheel@nd.edu
Duncan, Turner	PacBio	tduncan@pacificbiosciences.com
El Idrissi Saik, Imane	Hassan II University of Casablanca	imane.elidrissi.s@gmail.com
Elaagip, Arwa	University of Khartoum	arwalaagip@yahoo.co.uk
Ellis, David	UCL	david-ellis@ucl.ac.uk
Emrich, Scott	University of Tennessee (Knoxville)	semrich@utk.edu
Escalona, Hermes	CSIRO	hermes.escalona@csiro.au
Escalona, Merly	University of California Santa Cruz, California Conservation Genomics Project	mmescalo@ucsc.edu
Fablet, Marie	University Claude Bernard Lyon 1	marie.fablet@univ-lyon1.fr
Falcinelli, Monica	University of Camerino	monica.falcinelli@unicam.it
Fan, Zheng	Chinese Academy of Science	997827422@qq.com

Feder, Jeffrey	Univ. of Notre Dame	feder.2@nd.edu
Feitosa-Santos, Danillo Cristian	University of São Paulo	feitosa.dc@usp.br
Fernandez Goya, Lucia	FCEN - UBA	luluf.goya@gmail.com
Fisher, Cera	Cornell University	crf92@cornell.edu
Frederick, Julia	University of Georgia	julia.frederick@uga.edu
Ganatra, Akbar	International centre of insect Physiology and ecology	aganatra@icipe.org
Ganatra, Akbar	International centre of insect Physiology and ecology	akbar.ganatra@gmail.com
Garrigos, Marta	Estación Biológica de Doñana-CSIC	mgarrigosl@uoc.edu
Gasparotto, Ana Elisa	Sao Paulo State University - UNESP	ana.gasparotto@unesp.br
Ghassemi, Marzieh	Tehran University of Medical Sciences	ghasemimarzie@gmail.com
Giraldo-Calderón, Gloria I.	University of Notre Dame	ggiraldo@nd.edu
Giwa, Abdulazeez	Lagos State University	abdulazeez.giwa@lasu.edu.ng
Golday, Justin	Novogene Corporation Inc.	justin.golday@novogeneusa.com
Goldsmith, Marian	University of Rhode Island	mki101@etal.uri.edu
Gómez, Giovan F.	Universidad Nacional de Colombia - Sede La Paz	gfgomezg@unal.edu.co
Gomulski, Ludvik	University of Pavia	gomulski@unipv.it
Gonzalez, Juan	Univeristy of Notre Dame	jgonza34@nd.edu
Goubert, Clément	McGill University	goubert.clement@gmail.com
Grabowski, Jeffrey	Foundation for Advanced Education in the Sciences at the NIH	jgrabows25@gmail.com
Griffith, Katara	Virginia Tech	katarag@vt.edu
Grobler, Christiaan	University of Pretoria	cwgrobler@gmail.com
Guignard, Quentin	University of Pretoria, FABI	quentin.guignard@fabi.up.ac.za
Gulia-Nuss, Monika	University of Nevada, Reno; Depart	mgulianuss@unr.edu
Hackett, Kevin	USDA/ARS	kevin.hackett@usda.gov
Halfon, Marc	University at Buffalo	mshalfon@buffalo.edu
Hall, Jose	Milwaukee School of Engineering	hallj@msoe.edu
Halou, Daniel Kodjo	University of Ghana Medical School	dannyhalou@gmail.com
Hansen, Allison	University of California	allison.hansen@ucr.edu
He, Shulin	Chongqing Normal University	shulinhe@hotmail.com
Heckel, David	Max Planck Institute for Chemical Ecology	heckel@ice.mpg.de
Heu, Chan	ARS	chan.heu@usda.gov

Hinne, Isaac	University of Nevada	ihinne@nevada.unr.edu
Hira, Hasooba	University of Agriculture, Faisalabad, Pakistan	hasoobahira@gmail.com
Hodoameda, Peter	Kansas State University	hodoameda@ksu.edu
Holley, Jo Anne	University of Texas at Austin	jo.holley@utexas.edu
Howie, James	Boku	james.howie@boku.ac.at
Huang, Michelle	University of Notre Dame	mhuang6@nd.edu
Hui, Jerome	The Chinese University of Hong Kong	jeromehui@cuhk.edu.hk
Hull, Joe	USDA-ARS	joe.hull@usda.gov
Hunter, Wayne	USDA, ARS	Wayne.hunter@usda.gov
Jani, Viola	Institute of Public Health	violjani@gmail.com
Jiang, Haobo	Oklahoma State University	haobo.jiang@okstate.edu
Juma, Elijah	PAMCA	elijah.juma@pamca.org
Kachali, Kondwani	Malaria Alert Center	kondkachali@gmail.com
Kamatchi, Indumathi	Seri Biotech Research Laboratory	indumathi_28@gmail.com
Kamau, Joicy Luna Wangechi	Kenya Medical Research Institute	lkamau@kemri.org
Karega, Pauline	International Center for Insect Ecology and Physiology	karegapaul@gmail.com
Kelley, Melissa	University of Cincinnati	kelleym3@mail.uc.edu
Khan, Bilal	University of Agriculture, FSD, PAK	bilalentomologyuaf@gmail.com
King, Robert	Rothamsted Research	rob234king@gmail.com
Kioko, Grace	National Museums of Kenya	mwendegrace56@gmail.com
Kirstein, Oscar David	Emory	odkirstein@emory.edu
Kohli, Surbhi	University of Delhi	surbhikohli90@gmail.com
Koosha, Mona	Tehran University Of Medical SCiences	m.koosha@ymail.com
Kumar, Vikas	BITS Pilani	mosquitoresearcher@gmail.com
Kwak, Younghwan	University of California, Riverside	ykwak006@ucr.edu
Kwakye-Nuako, Godwin	University of Cape Coast	gkwakye-nuako@ucc.edu.gh
Lariviere, Patrick James (PJ)	University of Texas at Austin	pj.lariviere@utexas.edu
Larrosa Godall, Mireia	The Pirbright Institute	mlarrosa.6@gmail.com
Law, Sean Tsz Sum	The Chinese University of Hong Kong	seantslaw@link.cuhk.edu.hk
Lee, Hoi Ting	The Chinese University of Hong Kong	ivyleeting@yahoo.com.hk
Lerch, Anita	Univerity of Notre Dame	alerch2@nd.edu
Leskiewicz, Daniel	Milwaukee School of Engineering	leskiewiczd@msoe.edu

Li, Min	Jiangxi Normal University	LiM_hzpiuini@126.com
Li, Yiyuan	Ningbo University	lyy0005@gmail.com
Li, Zheng	University of Texas, Austin	zheng.li@austin.utexas.edu
Liang, Jiangtao	Virginia Tech	jtliang@vt.edu
Licata, Sophia	Indian River State College	licatase@mail.irsc.edu
Liu, Quanquan	University of Kentucky	qli259@uky.edu
Lopez-Rubio, Andres	Tecnologico de Antioquia	alopezru@tdea.edu.co
Love, Becca	University of North Carolina	rrlove@ad.unc.edu
Lown, Laurel	University of Notre Dame	llown@nd.edu
Lozada Chavez, Alejandro Nabor	University of Pavia	ALEJANDRO.CHAVEZ@UNIPV.IT
Lu, Hao-Ran	Chinese academy of science	luhaoran19@mails.ucas.ac.cn
Luan, Yun-Xia	South China Normal University	yxluan@scnu.edu.cn
Lukyamuzi, Edward	Pan-African Mosquito Control Association	edward.lukyamuzi@pamca.org
M T, RANJITH	Kerala Agricultural University	ranjith.mt@kau.in
Machado Medeiros de Albuquerque, Pedro	Universidade Federal do Rio Grande do Sul	albuquerque95pedro@gmail.com
Machingura, James	University of the Western Cape	3879081@myuwc.ac.za
Mack, Lindsey	University of California Davis	lindskay21@gmail.com
Maeda, Gerald (Jerry)	University of Texas at Austin	gmaeda17@utexas.edu
Maille, Jacqueline	Kansas State University	jmaille@ksu.edu
Makunin, Aleksei	Wellcome Sanger Institute	am60@sanger.ac.uk
Mann, Marina	Cornell University	mam734@cornell.edu
Manselle, Rachael	Alverno College	manselr@alverno.edu
Mao, Meng	University of Georgia	meng.mao@uga.edu
Marti, Emiliano	UNESP	mln.mrt@gmail.com
Martins Junqueira, Ana Carolina	Universidade Federal do Rio de Janeiro	anacmj@gmail.com
Massimino, Crissy	Indian River State College	emassimi@irsc.edu
Matoke-Muhia, Damaris	Pan African Mosquito Control Association	Damaris.matoke@pamca.org
Mbedzi, Pfano	University of Pretoria	Pfano.mbedzi@fabi.up.ac.za
McDowell, Mary Ann	University of Notre ame	mcdowell.11@nd.edu
Mejía Muñoz, Alejandro	University of Antioquia	alejo323mejia@gmail.com
Michels, Elizabeth	Indian River State College	Emichels2014@aol.com
Michels, Sarah	Indian River State College	smichels17@aol.com

Milani, Diogo	ICG	azafta@gmail.com
Mireji, Paul	Kenya Agricultural And Livestock Research Organization	mireji.paul@gmail.com
Mishra, Shaktiprasad	Odisha University of Agriculture and Technology	shaktiprasadmishra5@gmail.com
Mohamed, Nouh	Sirius Training and Research Centre	Nouh_saad@outlook.com
Molik, David	USDA ARS PWA PBARC	david.molik@usda.gov
Molina-Cruz, Alvaro	NIH	amolina-cruz@niaid.nih.gov
Mongue, Andrew	University of Edinburgh	amongue@ed.ac.uk
Mosqueda, Juan	Autonomous University of Queretaro	joel.mosqueda@uaq.mx
Mthawanji, Rosheen	Malawi Liverpool Wellcome Trust Clinical Research Programme	Caramel.mthawanji@gmail.com
Muleba, Mbanga	Tropical Diseases Research Centre	mbangamuleba@gmail.com
murithi, ken	International Centre of Insect Physiology and Ecology (icipe)	kenmurithi522@gmail.com
Mwangi, Jane	PAMCA	jane.njeri@pamca.org
Neiman, Alan	Indian River State College	Neimanat@mail.irsc.edu
Nanfack Minkeu, Ferdinand	The college of Wooster	fnanfackminkeu@wooster.edu
Naseem, Muhammad	National Institute for Biotechnology and Genetic Engineering	tayyibnaseem@gmail.com
Naseri karimi, Nazanin	School of public health, Tehran university of medical sciences	n-naserik@razi.tums.ac.ir
Navarro, Lucio	Cenicafe	lucio.navarro@cafedecolombia.com
Newton, Irene	Indiana University	irnewton@indiana.edu
Niu, Gengyun	Jiangxi Normal University	gyniu@126.com
Njoroge, Teresia	Indiana University School of Medicine	tenjorog@iu.edu
Nong, Wenyan	The Chinese University of Hong Kong	nongwenyan@cuhk.edu.hk
Norris, Laura	BMGF	laura.norris@gatesfoundation.org
Nowling, Ronald	Milwaukee School of Engineering	nowling@msoe.edu
Nsango Epse Tounda, Sandrine	University of Douala, Faculty of Medicine and Pharmaceutical Sciences and Centre Pasteur Cameroon	nsango2013@yahoo.fr
Ntege, Charles	Uganda National Malaria Control Division, MOH	charlesntege87@gmail.com
Ochomo, Eric	Kenya Medical Research Institute	ericochomo@yahoo.com
Ojo, James	Kwara State University, Malete	jamesadebayoojo@gmail.com
Olmstead, Morgan	USDA - ARS	Morgan.Olmstead@usda.gov
Oppenheim, Sara	American Museum of Natural History	soppenheim@amnh.org
Ortega, María	Museo de Historia Natural, UNMSM	maria.ortega@unmsm.edu.pe

Ortiz, Yasmin	IRSC	ortizy1@mail.irsc.edu
Oshaghi, Mohammad ali	Tehran University Of Medical SCiences	moshaghi@tums.ac.ir
O'Tousa, Joe	University of Notre Dame	jotousa@nd.edu
Owusu-Asenso, Christopher Mfum	University of Ghana	cowusu-asenso@st.ug.edu.gh
Panossian, Stephen	Md. Dept. Ag. Mosquito Control Section	Stephen.panossian@maryland.gov
Pedra, Joao	University of Maryland School of Medicine	jpedra@som.umaryland.edu
Pelloquin, Bethanie	London School of Hygiene and Tropical Medicine	bethanie.pelloquin1@lshtm.ac.uk
Peters, John	Milwaukee School of Engineering	petersjg@msoe.edu
Pfrender, Michael	University of Notre Dame	Michael.pfrender.1@nd.edu
PJ, Aneesha	Seri Biotech Research Laboratory	anuneena1998@gmail.com
Poelchau, Monica	USDA	monica.poelchau@usda.gov
Postma, Alisa	FABI, University of Pretoria	alisa.postma@fabi.up.ac.za
Rahman, Sarthok Rasique	The University of Alabama	sarthok@gmail.com
Ramasamy, Ellango	THSTI	ellango@thsti.res.in
Richards, Stephen	Baylor College of Medicine	stephenr@bcm.edu
Rolandelli, Agustin	University of Maryland, Baltimore	arolandelli@som.umaryland.edu
Roos, David	Univ Pennsylvania	droos@upenn.edu
Rose, Noah	Princeton University	noahr@princeton.edu
Rossi, Cristhian	Entomology Lab - UNMSM	cristhian.rossi@unmsm.edu.pe
Roy, Sonti	National Institute Of Animal Biotechnology	sontiroy@gmail.com
Ruiz Lopez, Maria Jose	Estación Biológica de Doñana-CSIC	mjruiz@ebd.csic.es
Rund, Samuel	University of Notre Dame	srund@nd.edu
Saha, Surya	Boyce Thompson Institute	ss2489@cornell.edu
Sakolvaree, Jira	USAMD-AFRIMS	JiraS.ca@afrims.org
Santiago, Paula Beatriz	Universidade de Brasília	paulabeatriz@hotmail.com
Schlieper, Alexis	Kansas State University	adschlieper@ksu.edu
Scully, Erin	USDA-ARS	Erin.Scully@usda.gov
Seck, Fatoumata	MRC-Gambia at LSTHM	seckafatoumata@gmail.com
Serrato, Idalba	INS	idalbamildred@gmail.com
Setton, Emily	UW Madison	setton@wisc.edu
Sewade, Wilfrid	Hacettepe Univ	sessowiles@gmail.com

Shafeeq, Tahir	University of Lahore	tahirshafeeq.uaf@gmail.com
Shank, Tim	Woods Hole Oceanographic Institution	Tshank@whoi.edu
Sharakhov, Igor	Virginia Tech	igor@vt.edu
Sharma, Prashant	University of Wisconsin-Madison	prashant.sharma@wisc.edu
Shaw, Dana	Washington State University	dana.shaw@wsu.edu
Shippy, Teresa	Kansas State University	tshippy@ksu.edu
Shivaprasad, Shwetha	Stanford Universiy	shwetha4@stanford.edu
Silva, Juan	Cornell University	js3274@cornell.edu
Silva, Rhavenna	Unesp	rhavennathais@hotmail.com
Singh, Upasana	University of Manchester	upasana.singh@manchester.ac.uk
Smith, Ashleigh	University of Pretoria	ashleigh.smith@up.ac.za
So, Wai Lok	The Chinese University of Hong Kong	wailokso@cuhk.edu.hk
Song, Hojun	Texas A&M University	hsong@tamu.edu
Sousa-Paula, Lucas	Oswaldo Cruz Foundation	lcsousapaula@gmail.com
Stahlke, Amanda	USDA-ARS	amanda.stahlke@usda.gov
Stanford-Beale, David	Purdue University	dstanfo@purdue.edu
Tank, Will	Kansas State University	wtank@ksu.edu
Tariq, Kaleem	AWKUM	kaleem.tariq@ufl.edu
Techer, Maeva	Texas A&M University	maeva.techer@ag.tamu.edu
Tims, Kelly	University of Georgia, Entomology Department	kelly.tims@uga.edu
Triant, Deborah	University of Missouri	triantd@missouri.edu
Tullume, Percy	USP	petullume@gmail.com
Ul Abdin, Zain	University of Agriculture, Faisalabad, Pakistan	zainentomology@uaf.edu.pk
Vasudevan, Swetha	Seri Biotech Research Laboratory	swjo270397@gmail.com
Vincent, Nitin	University of Notre Dame	nvincen2@nd.edu
Walker, William	USDA-ARS	william.walker@usda.gov
Walters, James	James Walters	jrwalters@ku.edu
Waterhouse, Robert	University of Lausanne and Swiss Institute of Bioinformatics	robert.waterhouse@gmail.com
Wei, Meicai	Jiangxi Normal University	weimc@126.com
Wei, Wen	Arizona State University	wwei39@asu.edu
Willis, Judith	University of Georgia	jhwillis@uga.edu
Wise, Tanner	Indian River State College	tfwise2000@gmail.com

Wolie, Zlanneu Rosine	Institut Pierre Richet (IPR)-VCPEC-IPR, Côte d'Ivoire/ Felix Houphouët Boigny (UFHB) University, Abi	wolierosine@yahoo.fr
Xu, Le	Zhejiang Provincial Center for Disease Control and Prevention	xule1208@zju.edu.cn
Yang, Yi	Zhejiang University	yylqy@zju.edu.cn
Yasmeen, Sumaira	Department of lifesciences	sumairagul11@hotmail.com
Ye, Xinhai	Zhejiang University	yexinhai@zju.edu.cn
Ye, Zhiqiang	Arizona State University	zhiqian7@asu.edu
Youngdech, Nittayaphon	USAMD-AFRIMS	NittayaphonY.ca@afrims.org
Zeng, Hua	CIBR	zenghua77@hotmail.com
Zhang, Mingpeng	Jiangxi Agricultural University	zhangmingpeng@outlook.com
Zhou, Xin	China Agricultural University	xinzhoucaddis@icloud.com
Zhu-Salzman, Keyan	Texas A&M University	keyan.zhusalzman@ag.tamu.edu

Notes:

Notes:

Notes: