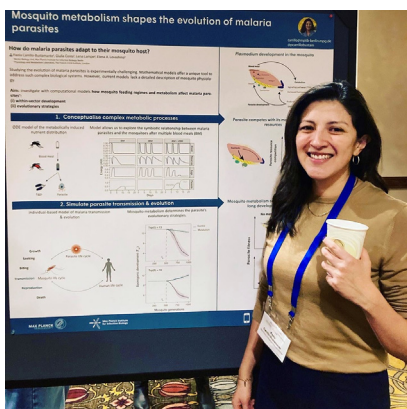


TrendsTalk

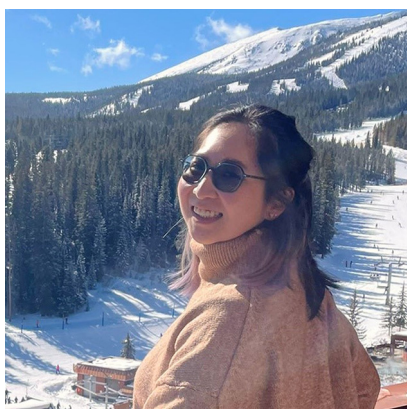
Keystone Vector Biology Symposium 2023: emerging concepts and novel technologies

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Keystone Symposia hosted a conference entitled ‘Vector Biology: Emerging Concepts and Novel Technologies’ on February 13–16, 2023, in Breckenridge, CO, USA. This meeting, co-organized by Joao H.F. Pedra (University of Maryland Baltimore, USA), Elena A. Levashina (Max Planck Institute for Infection Biology, Germany), and Maria-Carla Saleh (Institut Pasteur, France), emphasized the remarkable advances in vector-borne research by showcasing young scientists and researchers from around the world. To further foster connection across many different disciplines, the symposia consisted of joint sessions with the ‘Skin-Immune Crosstalk’ meeting held concurrently in Breckenridge. In this TrendsTalk, the organizing team highlight the novel findings presented at this meeting.



Paola Carrillo-Bustamante



Cassandra Koh

Keynote address

The Keystone Symposium began with the keynote address of Manu Prakash (Stanford University, USA), who introduced the principles of frugal science and illustrated its importance for community-driven engagement and surveillance of disease vectors at a planetary scale. Manu Prakash emphasized how broadening science and technology accessibility is essential for the benefits of novel inventions to reach their full potential. Thinking in this broader context requires the engagement of communities and an inclusive environment for collaborations between professional and amateur scientists, that is, health workers, and community managers: the people who are ‘the boots on the ground’. Manu Prakash elaborated on the low-tech technologies his laboratory has developed where citizen science has been impactful for vector biology. He started with Abuzz, a simple tool developed to map mosquito species worldwide. By using regular mobile phones, Abuzz measures mosquito wingbeat acoustics, yielding mosquito mapping data on a global scale at a wide spatiotemporal resolution. Manu Prakash presented next Vectorchip, an innovation to detect pathogens carried by mosquitoes in a field setting. This technology uses a rubber chip with microfluidics to perform biting assays. The amplification of mosquito DNA and viral RNA on these chips allows the quantification of viral copies deposited per bite. Since some salivary components are deposited in every bite, the mosquito species identity can be determined. This simple tool enables the monitoring of mosquitoes’ molecular signatures at a single-bite resolution. Manu Prakash showed SnapDx, an electricity free, saliva-based, at-home RTLAMP (reverse transcription loop-mediated isothermal amplification) tool for molecular diagnostics of diverse samples. Running diagnostics in a coffee cup, this tool has a cost of 30 cents for reagents per test, and 60 cents for hardware. He also presented Squid and Octopi, accessible and fully automated next-generation optical microscopy tools for malaria diagnostics in a blood smear. Finally, he mentioned Inkwel, an automated smear preparation as a simple mechanical solution to standardize the quality of blood smears for microscopy. Ultimately, Manu Prakash relayed the need for creating community-driven tools to democratize access to science, diagnostics, and ecological surveillance.





Anya J. O'Neal

Transgenesis, synthetic biology, and machine learning

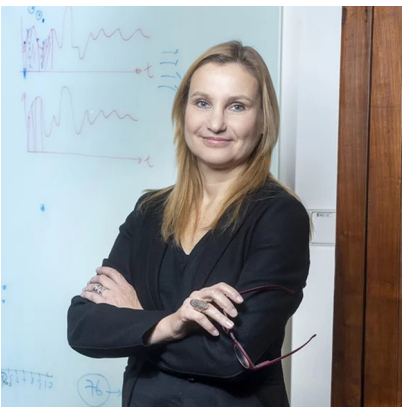
The first session was chaired by Monika Gulia-Nuss (University of Nevada, USA) and consisted of three fascinating talks outlining the advances made in transgenesis, synthetic biology, and machine learning for vector biology. Omar Akbari (University of California San Diego, USA) discussed the technologies developed in his laboratory in synthetic biology for mosquito research, including the progress and limitations of engineering *Aedes aegypti* mosquitoes resistant to dengue (DENV) and Zika viruses (ZIKV). Monika Gulia-Nuss emphasized how research in ticks lags behind that of mosquitoes, and revealed the techniques her laboratory has been developing to establish a novel embryo injection protocol and gene editing strategies for *Ixodes scapularis*. The final talk of the session was given by Felix Hol (Radboud University Medical Center, The Netherlands). He illustrated the potential of applying machine learning techniques to reveal key aspects of mosquito behavior and how it changes during viral infections. He displayed multiple tools developed in his laboratory that track mosquito movement, biting, and feeding behavior. He showed that infection with DENV enhances host contact, probing, and engorgement of *Ae. aegypti* mosquitoes.



Hanna J. Laukaitis

Immunobiology and metabolism 1

The first workshop session, led by Dana Shaw (Washington State University, USA), featured short talks on diverse aspects regarding vector immunobiology and metabolism. Patricia Scaraffia (Tulane University, USA) spoke about the regulation of nitrogen and carbon metabolism in female *Ae. aegypti* mosquitoes, specifically mitochondrial and cytosolic protein acetylation and how feeding regulates lysine acetylation. Agustin Rolandelli (University of Maryland Baltimore) characterized *I. scapularis* hemocytes by single-cell RNA sequencing and demonstrated that blood feeding influences hemocyte differentiation and proliferation. He also showed that the transcriptional signature changes upon blood feeding, inducing an enrichment in immunity, protein catabolism, cell growth, and molting genes. Oshani Ratnayake (Colorado State University, USA) compared the metabolic changes in *Ae. aegypti* induced by three different arboviral infections: DENV, ZIKV, and chikungunya virus (CHIKV), and illustrated how phospholipids and triglycerides are increased by all three viruses, especially at late time points of infection. Yu-Min Chuang (Yale University, USA) examined how cross-species signaling between mammalian adiponectin and mosquito adiponectin receptor impacts *Plasmodium* infection. Using a rodent malaria model, he demonstrated that both adiponectin depletion in the blood meal and knockdown of the adiponectin receptor in the mosquito increase *Plasmodium* infection intensity in the midgut. Paolo Gabrieli (Università degli Studi di Milano, Italy) presented mosquito saliva as a modulator of biting behavior, showing that the mosquito salivary protein LIPS-2 affects feeding behavior by triggering probing and salivation. Finally, Alan Goodman (Washington State University, USA) rounded off the session with a presentation on the role of insulin signaling in the context of arboviral infections. He demonstrated that insulin signaling induces a JAK/STAT-mediated antiviral response in adult mosquitoes and posited that insulin may drive arboviral evolution.



Elena A. Levashina

Microbial ecology and vector genetics

In this session chaired by Louis Lambrechts (Institut Pasteur), we were taken through the intrinsic and extrinsic factors governing arthropod vector competence for pathogens. Utpal Pal (University of Maryland College Park, USA) reports on a case of an evolutionarily conserved mammal–arthropod immune signaling crosstalk featuring the *I. scapularis* JAK/STAT receptor, Dome1. *Ixodes* spp. are highly diverse, yet genetically distinct



Maria-Carla Saleh



Joao H. F. Pedra

from other arthropods as is evidenced by the unique presence of a mammalian interferon-binding motif in its Dome1 receptor. Dome1 binds mammalian interferon-gamma to activate the tick JAK/STAT signaling pathway. The pathway activation promotes quicker blood meal acquisition, tick development, maintenance of gut biology, and surprisingly, higher levels of *Borrelia burgdorferi*. Following this, Louis Lambrechts himself presented an intriguing series of work on the genotypic interactions between ZIKV and its principal vector, *Ae. aegypti* mosquitoes, to explain why ZIKV outbreaks around the world are uniquely caused by the Asian lineage, even though the African lineage proved to be more transmissible in laboratory settings. The answer lies in the higher vectorial competence of non-African *Ae. aegypti* subspecies, *Ae. aegypti aegypti*, relative to its African counterpart, *Ae. aegypti formosus*. Shedding light on the enigmatic Cape Verde ZIKV outbreak, it was revealed that the local *Ae. aegypti* populations had higher levels of non-African ancestry. Shifting to a different vector species, Pamela Pennington (Universidad del Valle de Guatemala, Guatemala) condensed 30 years of work in developing a successful vector-symbiont intervention strategy to control Chagas' disease transmission by kissing bugs (triatomines) in Central and South America. Using a paratransgenesis approach, components of the triatomine bacterial microbiota were modified to produce double-stranded RNA targeting key *Trypanosoma cruzi* genes. In the next generation of this approach, to reduce vector populations, double-stranded RNA is being made to target triatomine genes. Moving the spotlight onto the viral microbiota of vectors, João Marques (Universidade Federal de Minas Gerais, Brazil) and his group unveiled the global virome profiles of *Ae. aegypti* and *Aedes albopictus* from 12 countries and highlighted two insect-specific viruses (incapable of replicating in vertebrate cells) that were most prevalent across the studied populations, Phasi Charoen-like virus and Humaita Tubiacanga virus. Investigating the impact of these two insect-specific viruses on DENV transmission, they found a positive effect mediated by histone gene expression. Paola Carrillo-Bustamante (Max Planck Institute of Infection Biology, Germany) elucidated how temperature and larval crowding stress shapes the population dynamics of the *Anopheles* mosquito vector. Her presentation highlighted the potential of multiscale modeling approaches built on empirical data in predicting the effects of climate on the evolution and transmission of *Plasmodium*.

Vector-borne pathogens at the skin interface

This first joint session of the Skin-Immune Crosstalk meeting was chaired by Nathalie Boulanger (Université de Strasbourg, France) and Sukanya Narasimhan (Yale University, USA), with a focus on how skin immune responses at the bite site modulates the infection success of vector-borne pathogens. Over the following talks, the concept of utilizing what we learn from vector-host interactions at the skin interface to facilitate the development of clinical tools is a common thread. Starting us off with trypanosomes transmitted by tsetse flies, Guy Caljon (University of Antwerp, Belgium) presented how the parasites increase infection success early on through a combination of transcriptomic modulation in the vector salivary glands and interactions with host dermal components and their immune response. Guy Caljon also explored the use of 'breathomics' to diagnose sleeping sickness in asymptomatic carriers based on volatiles associated with trypanosome infection in the lungs. Next, Sukanya Narasimhan dove into tick-host interactions to explain why ticks can feed multiple times with greater success on natural hosts than on non-natural hosts. In non-natural hosts, robust immune responses are induced at the bite site and tick feeding success is reduced after the first bite. This was revealed to be due to tick salivary proteins but also, to a significant extent, their associated

glycans, as the late physician-scientist and coauthor of the work, Dr Fred Kantor, The Paul B. Beeson Professor Emeritus of Medicine at Yale University, suspected. Collectively, this work brought us closer to a salivary-antigen-based vaccine against tick-borne pathogens. As Jesus Valenzuela [National Institute of Allergy and Infectious Diseases (NIAID), USA] illustrated, sandfly transmitted *Leishmania* parasites are a different beast as successful systemic dissemination of the parasite in the mammalian host is dependent on the regurgitation of sandfly gut bacteria at the bite site, resulting in recruitment of neutrophils, which the parasite preferentially infects. However, immunization with a recombinant salivary protein LJM19 lowers parasite load. In addition, he described the BITE study conducted to answer the question ‘Can the skin immune response to insect bites affect vaccine efficacy?’. Results revealed that naive patients, following multiple exposure to bites from *Ae. aegypti*, *Anopheles gambiae*, and *Lutzomyia longipalpis*, all displayed strong innate and adaptive immune responses at 48 h post-bite. Continuing with the notion that vector saliva enhances pathogen transmission, Julien Pompon (Institut de Recherche pour le Développement, France) and his team used metabolomics to explore whether lipids in mosquito saliva determine transmission success for mosquito-borne flaviviruses. Julien Pompon highlighted the role of certain phospholipids at stages of the virus replication lifecycle. Taking a different approach to a similar question, Hacène Medkour (Centre National de la Recherche Scientifique, France) described how single-cell RNA sequencing on skin cells from bite sites in a mosquito-to-mouse transmission model can be applied to study mammalian inflammation and interferon responses.

Microbiome, symbionts, and pathogens

The next session of day 2 was Workshop #2, which showcased short talks on the vector microbiome, symbionts, and pathogens, chaired by Eric Caragata (University of Florida, USA). Rebecca Johnson (Connecticut Agricultural Experiment Station, USA) presented her work on blood feeding dynamics and how this behavior affects DENV dissemination in *Ae. aegypti*. She demonstrated that multiple blood meals cause basal lamina damage in the midgut, which alters viral dissemination. Following this talk, Eric Caragata examined how larval competition stress and *Wolbachia* shape the fitness and viral infection of *Culex* mosquitoes. He showed that microbial competition impacts mosquito mortality, *Wolbachia* levels, and West Nile virus (WNV) infection. Next, Emily Gallichotte (Colorado State University, USA) observed how coinfection with St Louis encephalitis virus and WNV impacts replication, competition, and virus–virus interactions. Using samples from American robins and mosquitoes, two natural amplifying hosts, she found species- and tissue-dependent interactions between these viruses. Cassandra Koh (Institut Pasteur) spoke on the importance of reference rRNA sequences for improved metagenomics of sylvatic mosquito species. She explained that expanding the rRNA reference database for a wider range of mosquito species facilitates viral metagenomics, as it enables *in vitro* and *in silico* removal of rRNA reads during RNA sequencing analysis. Moving to ticks, Rachel Lange (University at Albany – State University of New York, USA) presented how *I. scapularis* plays a role in Powassan virus host specificity and adaptation. She showed that ticks contribute to viral diversity by driving the appearance of mutations in nonstructural proteins. Last, Fataimoudou Tandina (Université des Sciences Techniques et des Technologies de Bamako, Mali) revealed that *Plasmodium falciparum* infectivity is affected by mosquito feeding methods. She observed that direct skin feeding improves infectivity over membrane feeding, which may affect the mosquito’s midgut microbiota. Collectively, these short talks underscored the important relationships between vectors and the microbes they encounter.

Immunobiology and metabolism 2

The last session of day 2 was chaired by Meghan Hermance (University of South Alabama, USA) and highlighted research in vector immunobiology and metabolism. First, Elena Levashina (Max Planck Institute for Infection Biology) spoke on the Rel2/IMD pathway in *Anopheles* mosquitoes. Using tissue-specific transcriptomics, she observed that Rel2 is mainly active in the midgut and promotes mosquito survival after feeding. Carla Saleh (Institut Pasteur) examined the multifaceted contributions of mosquito Dicer2 to arbovirus transmission. She revealed how a Dicer2 mutation affects early viral replication and systemic viral dissemination of mosquitoes. Next, João Pedra (University of Maryland Baltimore) explained that ticks produce extracellular vesicles in their saliva that influence the skin immune environment. Using a multidisciplinary approach, he showed that extracellular vesicles promote tick feeding by regulating dendritic epidermal T cells in murine skin. Following these presentations, two speakers gave short talks on the arthropod IMD pathway. Anya O'Neal (University of Maryland Baltimore) explained that ticks possess a noncanonical IMD pathway and lack homologs for known receptors. She demonstrated that the *I. scapularis* homolog of croquemort elicits activation of the IMD pathway and restricts the acquisition of *B. burgdorferi*. Finally, Kevin Maringer (Pirbright Institute, UK) spoke on the *Aedes* IMD pathway and showed that it senses viral pathogen-associated molecular patterns (PAMPs). He also observed that a DENV protein suppresses this pathway in mosquitoes. Overall, these presentations highlighted the recent advancements in vector immunology and emphasized the need for further studies.

Skin-vector borne diseases: novel approaches to diagnosis, vaccines, and therapeutics

Saravanan Thangamani (State University of New York, USA) and Anne Moore (University of College Cork, Ireland) led the last day of the conference with a joint session involving novel approaches to diagnosis, vaccines, and therapeutics. The first speaker, Yasmine Belkaid (NIAID, USA), discussed her laboratory's contribution in understanding the influence of skin microbiota on cutaneous immunity. Using mouse models, her laboratory observed the role of the microbiota in regulating local antibody response independent of classical primary lymphoid tissues, such as the spleen, tissue repair, and neuronal regeneration. Next, Nathalie Boulanger (Université de Strasbourg, France) shared the current limitations in diagnosing Lyme borreliosis. Her talk focused on the detection of reliable markers indicative of active skin infection during both early and late stages of disease using proteomics of skin samples collected from human patients. Focusing on novel therapeutic approaches, Fiona Kuo (RAPT Therapeutics, USA) spoke about current mitigation techniques developed for the treatment of atopic dermatitis. This included targeting adaptive immune pathways through interleukin (IL)-4 and IL-13 signaling, modifying atopic dermatitis-associated cytokine responses, and lessening T cell migration by using CCR4 antagonists. With the advent of microneedles, Anne investigated the use of the 'ImmuPatch', which is a dissolvable microneedle patch. Work from her laboratory focused on the efficacy of administering vaccines to pigs at the skin interface through microneedles to circumvent the current limitations of vaccine distribution, including storage requirements, administration, cost, and availability. To complement this talk, Alexandra Francian (University of New Mexico, USA) discussed her work on determining components for virus-like particle vaccines. She focused on her progress in developing a combination vaccine containing both mosquito salivary proteins and malarial or viral antigens to increase protection against mosquito-transmitted diseases. Closing this joint session was Shrey A. Shah (University of

Maryland, College Park, USA), who works on developing microneedles as matrices to deliver molecular cargo into the skin. His talk featured monitoring skin immune reactions and cytokine release during application to investigate their adjuvant-like effect during delivery. These talks highlighted the monumental advancements in developing appropriate mitigation strategies for diseases at the cutaneous surface ranging across multiple disciplines.

Behavior, physiology, and reproduction

Our final session of the meeting, chaired by Geoffrey Attardo (University of California Davis, USA), focused on vector behavior, physiology, and reproduction. Diving into the mating behavior of mosquitoes, Leah Hour-Zeevi (Rockefeller University, USA) discussed how the evolution of female blood feeding is driven by mosquito genetics, nervous system, and behavior. Using behavioral assays, high-speed cameras, and transcriptomics, Leah described mosquito epigenetic regulation and hormone release as some factors associated with re-mating behavior in female mosquitoes. Next, Geoffrey Attardo spoke about the post-mating transcriptomic and metabolic profiles of tsetse flies. Utilizing a combination of technologies, his laboratory has uncovered several molecules related to the morphological changes that occur post-mating and factors shaping tsetse fly reproduction. The last speaker of the symposium was Kevin Macaluso (University of South Alabama, USA) who presented advances his laboratory has made with respect to *Rickettsia*-vector interactions. Specifically, he discussed uncovering the role of several bacterial-associated molecules involved in tick and flea transmission, as well as specific salivary components that facilitate flea feeding at the skin interface. These talks stress the importance of understanding the molecular components influencing behaviors of vectors responsible for the spread of diseases worldwide.

Closing remarks

Altogether, this meeting highlighted the vast array of vector biology research instilled at various institutions around the world. The talks presented herein document the significant strides the field has gained in developing cutting-edge technology that can be applied to vectors. They also emphasized the use of multidisciplinary approaches to understand disease ecology, vector biology, behavior, and pathogen-host interactions in nature. The emerging concepts evolving around skin-immune crosstalk mediated by protein and lipid exchange between arthropod vectors and hosts encouraged deeper collaborative efforts between skin biologists and entomologists. Finally, the highly attended poster sessions and extensive discussions during free time and meals contributed to the success of the Keystone Symposia meeting 'Vector Biology: Emerging Concepts and Novel Technologies'.

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