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## Meeting Report

### **The third International Workshop on *Aedes albopictus*: building scientific alliances in the fight against the globally invasive Asian tiger mosquito**

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From April 10–12 2017 scientists from around the world gathered in Pavia, Italy, to attend a workshop at the Palazzo Bellisomi-Vistarino hosted by the University of Pavia and dedicated to the latest research on the Asian tiger mosquito, *Aedes albopictus*. This biennial meeting was first convened in Pavia in March 2013 in response to the growing threat of this highly invasive mosquito species that can transmit several arboviruses of global public health significance including dengue, zika, and chikungunya viruses. Reflecting the native territories from which this mosquito has spread, the 2015 meeting was hosted by the Southern Medical University of Guangzhou, China. Returning to Europe in 2017, the meeting's scientific organizing committee was made up of Mariangela Bonizzoni and Giuliano Gasperi from the University of Pavia, who led the workshop, along with Andrea Crisanti from Imperial College, London (United Kingdom), Xiaoguang Chen from the Southern Medical University of Guangzhou (China), and Guiyun Yan from the University of California Irvine (United States of America). This report aims to inform the broader global health community of progress in *Ae. albopictus* research by providing several highlights of the results presented and discussed at the meeting, and to encourage participation in future workshops. The University of Pavia and the Southern Medical University of Guangzhou have signed a joint agreement to maintain support for running this workshop biennially between the two universities.

The first session focused on mosquito surveillance and intervention strategies, with reports from the African, American, Asian, and European perspectives. Xiaoguang Chen opened the meeting with an evaluation of methodologies and techniques used for surveillance in China, including comparisons of the efficiency of oviposition traps, CDC (Centers for Disease Control and Prevention) light traps and BG (Biogents) traps which indicated that BG traps are best suited for *Ae. albopictus* in China. Alessandra della Torre presented data showing that the number of eggs in oviposition traps commonly used to monitor species abundance only roughly correlates with adult abundance, and revealed promising results from a pilot community engagement initiative designed to help monitor mosquito contact with humans (the online and mobile application ZANZAMAPP, [www.zanzamapp.it](http://www.zanzamapp.it)). Using data from BG traps for model calibration, Roberto Rosà described the mathematical modelling of the temporal dynamics of *Ae. albopictus* and *Culex pipiens* in northern Italy and the important effects of interspecific competition. Recent progress in the application of sterile insect technique (SIT) programs in Italy were outlined by Romeo Bellini, with a focus on efforts to improve methods for sex separation and quality control in routine mass rearing of *Ae. albopictus*. Maurizio Calvitti presented data on the effectiveness of using an *Ae. albopictus* strain (ARwP) with altered *Wolbachia* infection for *Ae. albopictus* population suppression and discussed the possible advantages in incompatible insect technique (IIT) strategies deriving from a bidirectional incompatibility pattern. *Wolbachia* based IIT approaches against *Aedes aegypti* developed as part of the Debug Project at Verily Life Sciences were outlined by Jacob Crawford, as well as plans to assemble a genome database with population samples from around the globe. Margareth Capurro presented lessons from the Brazilian mass rearing and community awareness efforts for the control of *Ae. aegypti*, examining combined SIT and IIT approaches as well as a transgenic based approach using RIDL (Release of Insects carrying a Dominant Lethal gene). Frederic Simard reported that in Central Africa *Ae. albopictus* is spreading from urban areas to forests bringing a concomitant risk of the emergence of new zoonotic arboviruses – understanding this threat is a key motivation of the recently launched TIGERBRIDGE research project.

Session two focused on advances in *Aedes* genomics, where several efforts have been hampered by the large sizes and repeat-rich nature of their genomes. William Black examined the limits of unassembled genomes, especially when performing Genome Wide Association Studies (GWAS), but showed how using an improved *Ae. aegypti* assembly (AegL4) to investigate deltamethrin resistance identified a clear selective sweep in the region of the *voltage gated sodium channel* gene and its neighboring genes. Presenting quantifications of the completeness of available genome assemblies and annotations using universal single-copy orthologues, Robert Waterhouse outlined the current status of *Aedes* genomics resources and called for efforts to improve genome assemblies and share transcriptomics data through VectorBase ([www.vectorbase.org](http://www.vectorbase.org)). Maria Sharakhova explained how physical genome mapping data are vital to improve draft genome assemblies, revealing that mitotic chromosomes from imaginal discs of *Ae. albopictus* have banding patterns suitable for mapping scaffolds to chromosomal arms and presenting data on aberrant karyotypes from the *Ae. albopictus* C6/36 cell line. While sex-specific DNA sequences in *Ae. albopictus* remain elusive, Igor Sharakhov demonstrated species-specific differences in the structures of X- and Y-heterochromatin and chromosomal abnormalities in F1 hybrids of anopheline mosquitoes that advance our understanding of the mechanisms of male sterility and their implications for the applications of SIT approaches.

The third session was dedicated to population genetics and genomics of *Aedes* mosquitoes. Highlighting key lessons from studies on *Ae. aegypti*, Jeffrey Powell offered insights to help guide future *Ae. albopictus* research including on the relative merits of using mitochondrial DNA, microsatellites, restriction site

associated DNA (RAD) sequencing, and single nucleotide polymorphism (SNP) chip data for population genetics and GWAS. Using *Ae. albopictus* microsatellites to investigate global genetic variation and population structures, Giuliano Gasperi presented alternative hypotheses on possible colonization histories with the data indicating a recent and chaotic human-mediated propagule worldwide dispersion pattern, and results from analyses connecting the co-ancestry (genetic clustering) of populations with their degree of virus transmission efficiency. Gisella Caccone's results from phylogeographic studies with a panel of SNPs from 20 worldwide invasive and native populations supported a history of multiple invasions or colonization by a large number of propagules, and provided estimates of linkage disequilibrium levels similar to tsetse flies and higher than anophelines.



Figure 1. Workshop participants at the Palazzo Bellisomi-Vistarino, Pavia, Italy.

Session four showcased advances in ecological studies and adaptation genomics. Peter Armbruster shared his results from investigating the roles of *Ae. albopictus* small RNAs and metabolites in diapause regulation: they indicate that microRNAs appear to be important at the stage of developmental arrest in the egg but do not contribute to diapause regulation in mature oocysts, and they reveal several catecholamines that are differentially abundant in diapause and non-diapause eggs and are related to juvenile hormone regulation. Transcriptome sequencing of female antennae and maxillary palps together with extensive comparative genomic analyses identified a comprehensive catalog of the *Ae. albopictus* olfactory repertoire presented by Fabrizio Lombardo, including odorant binding proteins (OBPs), odorant receptors (ORs), ionotropic receptors, and gustatory receptors. Focusing on differences amongst wild populations, Ludvik Gomulski described the results from the comparative analysis of *Ae. albopictus* antennal transcriptomes from Thailand, Greece, and Italy, which identified differentially expressed genes and a large panel of SNPs including many in OBPs and ORs that may be linked to local adaptations.

The fifth session examined interactions between vector mosquitoes, viruses and other microbes. Focusing on the variable vectorial competence of *Ae. albopictus* populations, Anna-Bella Failloux examined viral genetic diversity from outbreaks worldwide and suggested that, similar to dengue and chikungunya, adaptation by zika could result in local transmission in Europe. Dimitri Lavillette's report on dengue-chikungunya co-infections addressed the paucity of detailed knowledge on how such

infections in mosquitoes may influence the pathologies. His results indicate that dengue infections were enhanced in cells pre-infected with chikungunya and that this phenomenon can be generalized to other flaviviruses, paving the way for characterizing Flavivirus-Alphavirus molecular interactions and a better understanding of the epidemiology of simultaneous outbreaks. Mariangela Bonizzoni presented results from comprehensive searches of more than 20 mosquito genome assemblies for sequences of RNA viruses, showing that viral integrations are not uniformly distributed among species, but are particularly abundant in *Aedes* mosquitoes, where they are enriched in piRNA clusters and produce piRNAs. These results suggest that viral integrations depend not only on virus exposure, but also on specific virus-host interactions, and that they represent an un-appreciated source of genome variability and confer heritable immune signals. By profiling small RNAs, Ronald Van Rij showed that *Ae. aegypti* and *Ae. albopictus* produce virus-derived piRNAs in response to infections and host-derived piRNAs from transposon or protein-coding gene sequences as well as from endogenous integrated viral elements. Jean-Luc Imler described a recently-developed metagenomics strategy based on small RNA sequencing for surveying viral sequences in insects, which revealed Phasi Charoen-like virus, Humaita Tubiacanga virus, and a previously unknown Mosquito Caratinga virus from mosquitoes captured in a dengue endemic urban area in Southeast Brazil. By examining interactions between the mosquito's RNA silencing machinery and the yellow fever virus capsid protein, Kevin Myles demonstrated the ability of the virus to antagonize the mosquito's antiviral responses by interfering with Dicer activity. Starting from the human RNA helicase DDX3X that is known to inhibit replication of viruses including West Nile and dengue, Giovanni Maga described an *Ae. albopictus* ortholog (called *Belle* in *Drosophila*) and the rationale for targeting this protein in strategies to control Flavivirus replication in mosquitoes. Patrick Mavingui presented the brand new world-class mosquito biosecure research facilities for experimental infection and innovative control development in La Reunion Island and discussed results from genomic profiling to investigate the contribution of the associated bacterial microbiota to *Ae. albopictus* adaptation and competence. Guido Favia described metagenomic analyses of the microbiota associated with different organs of *Ae. albopictus* adults to identify promising targets for use in future control strategies and showed how metabolites produced by the acetic acid bacterium *Asaia* may be able to interfere with zika infection in mosquito cells.

The concluding session of the meeting was dedicated to the newly-launched INFRAVEC2 research project (<https://infravec2.eu>), presented by Anna-Bella Failloux and Frederic Simard. This important project is an international and interdisciplinary initiative funded to the tune of 10M€ by the Horizon 2020 Research Infrastructure Program of the European Union. It focuses on insect vectors of human and animal disease, including mosquitoes, sandflies and other flies, and is coordinated by Kenneth Vernick from the Pasteur Institute in Paris. It follows on from the first INFRAVEC project and aims to continue to develop research resources and provide access to key infrastructures for insect vector biology at no cost to the end-user, available through an online catalogue. The 24 project partners host the major European biosecure insectaries for experimental infection and containment of insect vectors, other key insect vector facilities, and include front-line field sites in Africa, the Pacific, and the Americas. The development of new resources, standardizing of laboratory protocols, and the ability to share access to large infrastructures will no doubt substantially help to advance *Ae. albopictus* research in Europe and beyond.

Presentations were complemented by a selection of posters covering different biological research themes for *Ae. albopictus* and other insects. These included: Progress towards the development of serological markers of human exposure to *Aedes* mosquitoes using *Ae. albopictus* salivary antigens. A case study from Rome for estimating the probability of arbovirus outbreaks in non-endemic countries highly

infested by *Ae. albopictus*. Establishing a C-Type Lectin-3 (GCL-3) mutant mosquito using the CRISPR/Cas9 system. Detailed investigations of *Ae. albopictus* olfactory proteins and comparisons with other insects. The identification of new bacterial species with insecticidal activities towards *Ae. albopictus* larvae. Orthology-based genome annotation and interpretation to leverage comparative genomics to build detailed hypotheses on gene function. Investigating long-term signatures of insectary adaptation in *Ae. albopictus*. Exploring the composition and function of the tsetse fly ejaculate. And a comprehensive overview of the Liverpool Insect Testing Establishment (LITE).

Overall, the workshop provided a dynamic forum for in depth discussions on the challenges and opportunities facing both *Ae. albopictus* fundamental biological research and the ultimate goals of comprehensive surveillance of vectors and pathogens as well as multi-faceted effective mosquito control programs. Some of the greatest leaps forward that have been made since the first workshop in 2013 stem from the new genomic resources developed over the last few years, and undoubtedly the interactions amongst the participants will have stimulated new and exciting approaches to harness these and other resources to tackle the global threats posed by the Asian tiger mosquito.

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