

ABSTRACT BOOK



Montpellier, France
May 11-13, 2022

5th International Workshop on *Aedes albopictus*

Montpellier

May 11-13, 2022



Dear participants to the 5th international workshop on *Aedes albopictus*, 11-13 May 2022, Montpellier, France

On behalf of the organizing and scientific committees, I am extremely pleased to welcome you all in Montpellier France, the mosquito paradise or mosquito hell, depending on the point of view of researchers, operators, public health decision makers, or of mosquitoes.

I sincerely thank all participants for contributing to this edition through talks, posters, ideas, suggestions. I hope this meeting will be bad news for the tiger mosquito, demonstrating that we are progressing in the control of this mosquito and the diseases it transmits.

I would also like to warmly thank our generous sponsors who have allowed this workshop to take place for the first time in France, under very favorable conditions: the University of Montpellier, the Occitanie Region, the Metropolis of Montpellier.

I would also like to thank our local committee, including Elodie Suttling, Hanna Emlein, Morgane Henard, and many others in the background, for the organization of this colloquium.

I ask for your indulgence regarding small issues that may arise. We are at your disposal should you require any assistance during your stay.

Enjoy this meeting as much as possible!

Didier Fontenille, IRD - University of Montpellier, on behalf of both committees



5th International Workshop

on *Aedes albopictus*

the Asian tiger mosquito

May 11-13, 2022

Montpellier, France

Final Agenda

Wednesday, May 11, 2022

9h00: Welcome address

- Mariangela BONIZZONI, Workshop scientific committee representative
- Didier FONTENILLE, IRD, Montpellier University: *Aedes albopictus*: How far will it harm humans? (5mn)
- Clare HART, Vice-Présidente Montpellier Méditerranée Métropole, Déléguée aux Relations Internationales et à la Coopération Européenne
- Catherine CHOMA, Directrice de la santé publique, ARS Occitanie, French Ministry of Health
- Vice-Présidente Recherche, Région Occitanie, or representative
- President/ Rector of the University of Montpellier or representative

9h30 – 10h00: First keynote address by **Jeffrey Powell** (Yale University - USA): “What *Aedes aegypti* can tell us about *Aedes albopictus*?”

Session 1: new advances in phylogeny, evolution, genetics and genomics of *Aedes albopictus*

Chairpersons: Mariangela Bonizzoni, Pavia University, Italy., Claire Valiente Moro, University of Lyon, France

10h00 – 10h40

1. Maria Sharakhova, United States: Chromosomal rearrangements in aedini mosquitoes, including *Aedes albopictus*
2. Sébastien Boyer, Cambodia: A new Asian tiger mosquito species in Cambodia

10h40 - 11h10: Break

11h10- 12h15

3. Andrea Gloria-Soria, USA : Population genetics of an invasive mosquito vector; *Aedes albopictus* in the Northeastern USA
4. Philippos Papathanos, Israel: CRISPR Developing genetic engineering tools for the tiger mosquito
5. Adalgisa Caccone, USA: A SNP chip for *Aedes albopictus*

13h30 – 14h00: 2nd Key note address by **Ronald Van Rij** - Radboud University Medical Center – Netherlands: Small RNAs at the nexus of gene regulation, development and antiviral defense in *Aedes*

Session 2: Virus-vector interactions

Chairpersons: Annabella Failloux, Institut Pasteur, Paris ; Alto Barry, University of Florida, USA

14h00- 15h50

1. Gasmi Laila, Italy: Antiviral potential of *Aedes albopictus* non-retroviral endogenous viral elements
2. Maria Vittoria Mancini, Italy: *Wolbachia* strain wAu efficiently blocks arbovirus transmission in *Aedes albopictus*
3. Pascal Miesen, Netherlands: Arbovirus - mosquito interactions: Zooming in on RNA
4. Trindade Marques Joao, Brazil: Comparative analysis of the global virome of *Aedes albopictus* and *Aedes aegypti* mosquitoes
5. Anna Malacrida, Italy: Provenance and competence in *Aedes albopictus*

15h50 – 16h20: Break

Session 3: Development, Physiology, ecology, Behaviour of *Aedes albopictus*

Chairpersons: Isabelle Kramer, Goethe University Frankfurt, Germany; Christophe Paupy, IRD, Montpellier.

16h20- 18h10

1. Isabelle Kramer, Germany: The ecophysiological plasticity of invasive *Aedes* mosquito species - a comparative study of global, South Asian and European populations
2. Judicaël Obame-Nkoghe, Gabon: Invasion of *Aedes albopictus* in forests of Central Africa: zoom on the situation observed in the National Parc of La Lopé (Gabon)
3. Ruth Müller, Belgium: Vector biology of *Aedes albopictus* and eco-bio-social drivers In the Himalaya-Hindukush region
4. Claire Valiente Moro, France: Mosquito sex and active mycobiota contribute to fructose metabolism in the Asian tiger mosquito *Aedes albopictus*
5. Federico Forneris, Italy: A salivary factor binds a labrum cuticular protein and controls biting in mosquitoes

18h45 – 21h00: Cocktail. Venue : Salle Pétrarque, historical city center

Thursday, May 12, 2022

8h45 – 9h15: 3rd Key note address by **Omar Akbari** - University of California San Diego - USA: Using Precision guided sterile insect technique (SIT) to suppress mosquito populations

Session 4: surveillance (distribution, resistance) of *Aedes albopictus*

Chairpersons: Annelise Tran, CIRAD, France; Francis schaffner, Consultant, Switzerland

9h15- 10h45

1. Annelise Tran, France: ARBOCARTO: an operational spatial modeling tool to predict *Aedes albopictus* dynamics and the impact of vector control interventions
2. Roger Eritja Spain: Citizen-based surveillance of invasive mosquitoes within multi-sourced modelling
3. Francis Schaffner Switzerland: Continental-scale surveillance of *Aedes albopictus*: standardisation for moving from distribution to abundance
4. Jean-Philippe David, France: TigRisk: Evaluating the risk of insecticide resistance in the tiger mosquito: A predictive approach combining field surveillance, experimental selection and molecular markers

10h45 – 11h15: Break

Session 5 (part 1): *Ae. albopictus* control tool development and field evaluation

Chairpersons: Alessandra della Torre, University La Sapienza, Italy ; Charles Wondji, CRID, Yaounde, Cameroon

11h15- 13h00

1. Alto Barry, USA: Lethal and nonlethal effects of insecticides in *Aedes albopictus*
2. Maurizio Calvitti, Italia: SIT and IIT to tackle *Aedes* invasive mosquitoes: conjugating efficiency and economic sustainability
3. Jérémy Bouyer, France / Austria: New developments in the use of the Sterile Insect Technique against *Aedes albopictus* in Europe
4. Louis Clement Gouagna, France: Pilot control trial by releases of sterile males against *Aedes albopictus* in Reunion Island
5. Grégory L'Ambert, France: Autodissemination method against *Aedes albopictus* in a field experiment

13h00 – 13h15: Group Photo

13h15 - 14h15: Lunch at the Corum

14h15 – 14h45: 4th Key note address by **Charles Wondji** - Liverpool School of Tropical Medicine – Center for research in Infectious Diseases - Cameroun: *Aedes albopictus* insecticide Resistance

session 5 (part 2): *Ae. albopictus* control tool development and field evaluation

14h45 – 15h45

6. Carla Sousa, Portugal: Insecticide resistance and population genetic analysis of *Aedes albopictus* populations in mainland Portugal
7. Barnabas Zogo, Sumitomo Chemical, UK: A review of pyriproxyfen-based larvicides against *Aedes albopictus* and an introduction to a new long lasting formulation of pyriproxyfen for use in containers and storage vessels
8. Carine Ngoagouni, Central African Republic: Insecticide resistance in *Aedes albopictus* in Central Africa and the Prospects for control management

15h45 – 16h15: Break

Session 6: Public Health perspectives

Chairpersons: Eleonora Flacio, University of applied sciences of Southern Switzerland;
Omar Akbari, UCSanDiego, USA

16h15 – 17h45

1. Guillaume Lacour, France: The quest for relevant indicators, evidence of effectiveness and improvement of Vector Control in France from 2010 to 2021
2. Franck Remoue France: Innovative biomarker of human exposure to *Aedes* bites: from concept to applications to the assessment of the risk of arbovirus transmission and vector control efficacy
3. Knols Bart, Maldives: Mass trapping and larval source management for the elimination of *Aedes albopictus* from small tropical islands
4. Marie claire Paty, France: *Aedes albopictus* in France: Lessons learned and future challenges for surveillance and prevention, the French experience

17h45- 18h00: Conclusions of the basic research sessions

Friday, May 13, 2022

9h00 – 9h30: 5th Key note address by Alessandra Della Torre, Sapienza University - Italy:
“AIM Cost results, lessons and messages”

Session 7: Public health concerns, management of control, market point of view

Chairpersons: Carla Sousa, IHMT, Portugal ; Guiliano Gasperi, Pavia University, Italy

09h30 – 10h15

1. Florence Fouque, TDR/WHO, Switzerland: The WHO response against *Aedes albopictus* through the WHO Global Vector Control Response (GVCR), the WHO Global Arboviruses Initiative (GAI) and the TDR/WHO supported activities
2. Vincent Corbel, France: INOVEC: a Research & Innovation Partnership for enhancing the surveillance and control of mosquito vectors of emerging arboviruses

10h15 – 10h45: Break

10h45 – 11h45

3. Clelia Oliva, XperTIS, France: Bringing SIT to market in France: towards a more efficient control of the tiger mosquito
4. Yvon Perrin, FRANCE: Vectrap: Applicability of mass trapping strategy against *Aedes albopictus* and *Aedes aegypti*

12h00 - 13h30: Lunch at the Corum

Session 8: *Aedes albopictus* control in Europe: what is missing? Learning from other territories

Chairpersons: Florence Fouque, WHO, Switzerland; Roger Eritja , CREA, Universitat Autònoma de Barcelona, Spain

13h30 – 15h00

1. Olivier Briet, ECDC, Sweden: Wanted: Cost-effectiveness of *Aedes albopictus* control strategies
2. Eleonora Flacio, Switzerland: *Aedes albopictus* surveillance and control system (2000-2022): strategies and results in Switzerland

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3. Mattia Calzolari, Italy: Monitoring data supporting larval control in urban areas of Emilia-Romagna, Italy
4. Sarah Delacour, Spain: *Aedes albopictus* surveillance system – Spanish experience

15h00 – 15h30: Break

15h30 – 16h30: Discussion and conclusion

Chairpersons: Didier Fontenille and Scientific committee

END OF WORKSHOP

5th International Workshop on *Aedes albopictus*

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Session 1: New advances in phylogeny, evolution, genetics and genomics of *Aedes albopictus*

Chairpersons:

Mariangela Bonizzoni, Pavia University, Italy

Claire Valiente Moro, University of Lyon, France

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Keynote address 1: What *Aedes aegypti* can tell us about *Aedes albopictus*?

Jeffrey Powell, Yale University, USA

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Aedes aegypti and *Aedes albopictus* have remarkable parallels in their history and in the threats they pose to human health. Both are ultimately derived from ancestral *Stegomyia* in Asia. The major difference is that *aegypti* evolved into a human commensal and spread around the world about 500 years ago while *albopictus* only started expanding about 75 years ago. The accumulation of worldwide population genetic databases have allowed us to both understand the history and to identify origins of new introductions of *aegypti*. We have also tried to determine the genetics of variation in populations of *aegypti* in the ability to transmit arboviruses with limited success. This work should help guide similar studies in *albopictus*.

OC 1.1: Chromosomal rearrangements in aedini mosquitoes, including *Aedes albopictus*

Maria Sharakhova, Virginia Polytechnic and State University, USA

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Chromosomal inversions have been shown to play an important role in mosquito evolution. In this study, we utilized Hi-C-capturing approach to discover chromosomal rearrangements in 22 strains of the dengue vector *Aedes aegypti* from different world-wide locations. We found 20 multi-megabase chromosomal inversions with sizes varied from 55 to 1.5 Mb (~20 Mb average). The presence of inversions was validated by fluorescence in situ hybridization in mitotic chromosomes. Inversions were unevenly distributed along the chromosomes and were more abundant in 1q and 3p arms which are homologous to the inversion-rich 2R chromosomal arm in malaria vector *Anopheles gambiae*. The highest number of inversions was found in Western Africa. In addition, we developed a physical genome map for the most dangerous invasive vector of arboviruses *Aedes albopictus*. The mapping discovered at least 4 large chromosomal rearrangements between *Ae. aegypti* and *Aedes albopictus*. The results suggest an existence of a large pool of structural variations in aedine mosquito genomes potentially involved in adaptations and pathogenesis.

OC 1.2: A new Asian tiger mosquito species in Cambodia

Sébastien Boyer, Institut Pasteur du Cambodge, Cambodia

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Correct species identification of mosquito-borne diseases vectors is crucial to implement vector control strategies. In Cambodia, a sampling effort was done to collect *Aedes* specimens belonging to the subgenus *Stegomyia*, which includes notorious vector species of dengue, zika and chikungunya. In addition to their morphological identification, Cytochrome c oxidase subunit 1 (cox1)

gene from the mitochondrial DNA was sequenced for molecular characterization and genetic analysis. Molecular analysis with strong bootstrap values of 36 *Ae. albopictus* *cox1* sequences confirmed morphological identification. However, 14 specimen morphologically identified as *Ae. albopictus* revealed divergent *cox1* sequences. This group was further investigated for rDNA 5.8S-ITS2 sequencing and for Wolbachia presence. The rDNA 5.8S-ITS2 sequences confirmed a cluster different from *Ae. albopictus* and the mosquitoes from this new OTU were negative for Wolbachia presence, contrary to *Ae. albopictus*, which carries wAlbA and wAlbA B types. Therefore, this group is considered to be a new Operational taxonomic unit (OTU).

OC 1.3: Population genetics of an invasive mosquito vector; *Aedes albopictus* in the North-eastern USA

Andrea Gloria-Soria, The Connecticut Agricultural Experiment
Station, USA

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The Asian tiger mosquito (*Aedes albopictus*) arrived in the USA in the 1980's and rapidly spread throughout the country within a decade. The predicted northern edge of its overwintering distribution on the East Coast of the USA roughly falls across New York, Connecticut, and Massachusetts, where the species has been recorded as early as 2000. It is unclear whether *Ae. albopictus* populations have become established and survive the cold winters in these areas or are recolonized every year. We genotyped and analyzed populations of *Ae. albopictus* from the northeast USA using 15 microsatellite markers and compared them with other populations across the country and to representatives of the major global genetic clades to investigate their connectivity and stability. Overall, our results support the presence of established populations of *Ae. albopictus* in New York, Connecticut, and Massachusetts, successfully overwintering and migrating in large numbers. Given the stability and interconnectedness of these populations, *Ae. albopictus* has the potential to continue to proliferate and expand its range northward under mean warming conditions of climate change.

OC 1.4: Developing genetic engineering tools for the tiger mosquito

Philippos Papathanos, Hebrew University of Jerusalem, Israel

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Doron Zaada¹, Vytutas Mackevicius¹, Dor Perets¹, Guy Ostrovsky¹, Eliezer Scharlat¹, **Philippos Aris Papathanos**¹

¹ Department of Entomology, Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Rehovot, Israel

Invasive species pose widening epidemiological, agricultural and biodiversity threats globally. Among them, the Asian Tiger mosquito *Aedes albopictus* is the most aggressive and invasive mosquito species, and is a major vector for arboviruses such as Dengue, Zika and Chikungunya Fever. Modern genetic editing capabilities hold great promise for developing novel tools for both research and control. However, compared to other mosquito species, gaps in knowledge and tools for *Aedes albopictus* genetics and genomics have received little attention. In an effort to close these gaps, we are now developing genetic engineering tools in this mosquito, from endogenous genomic components for transgene regulation to improved designs that enable precise genome editing and/or genetic control. We will present data on the creation of novel genetic transformation markers that can now increase the overall number of transgenes that can be easily identified within a single insect. We will also present progress on the development of reverse genetic tools for exploring gene function in this mosquito, including new recessive mutant strains for evaluating *in vivo* somatic and germline mutagenesis rates, and constructs for transgene-enhanced CRISPR genetic engineering.

OC 1.5: A SNP chip for *Aedes albopictus*

Gisella Caccone, Yale University, USA

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I describe the procedure we used to develop a Snp chip for *A. albopictus* and preliminary results to evaluate the chip quality. SNPs for tiling on the chip were identified using 819 WGS data from 42 worldwide populations. We used sites with at least a10X coverage in all populations, obtaining ~ 34 million polymorphic sites. After filtering we had ~2 million high-quality bi-allelic polymorphic sites. The Affymetrix bioinformatics pipeline selected ~1,2 million SNPs for chip design. Next, we selected 250,000 markers across the genome based on their location and distance. Affymetrix was able to tile 175,396 on the chip. We obtained approximately 102 SNPs per 1Mb window. We then used five chips to genotype single mate crosses and wild mosquitoes from different geographical regions to validate the chip and provide preliminary analyses on its ability to detect genetic structuring and comparison with the 175K SNP set from the WGS data.

Session 2: Virus-vector interactions

Chairpersons:

Annabella Failloux, Institut Pasteur, Paris, France

Alto Barry, University of Florida, USA

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Keynote address 2: Small RNAs at the nexus of gene regulation, development and antiviral defense in *Aedes*

Ronald Van Rij, Radboud University Medical Center, The Netherlands

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In animals, three RNA silencing pathways exist in which a small RNA guides an Argonaute family protein to target RNAs to induce silencing. Of these, the PIWI interacting RNA (piRNA) is the least well understood. Work in genetic model organisms such as the fruit fly *Drosophila melanogaster* indicates that the piRNA pathway is essential for genome integrity by suppressing transposable elements in the germline. However, the piRNA pathway in the vector mosquito *Aedes aegypti* and *Aedes albopictus* is characterized by an expanded, broadly expressed PIWI gene family, suggestive of new functions beyond transposon control. Indeed, piRNAs in *Aedes* are produced from several non-canonical substrates, including viral RNAs, protein-coding genes, satellite repeats and endogenous viral elements (EVEs), sequences derived from non-retroviral RNA viruses that are integrated in mosquito genomes. I will discuss our recent insights into the function of piRNAs in gene regulation, early embryonic development and antiviral defense.

OC 2.1: Antiviral potential of *Aedes albopictus* non-retroviral endogenous viral elements

Laila Gasmi, University of Pavia, Italy

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The genome of the mosquito *Aedes albopictus* harbours hundreds of non-retroviral Endogenous Viral Elements (nrVEs). Most of these nrVEs encompass fragmented viral open reading frames and are embedded within piRNA clusters. I identified nrVEs that map outside piRNA clusters, include functional motives typical of RNA dependent RNA polymerases (RdRPs) and are transcribed at the mRNA level. RdRPs are viral genes that mediate RNA virus replication and translation; in eukaryotes, RdRPs have been described and functionally validated as part of the antiviral RNA interference pathway in plants, fungi and nematodes. Additionally, RdRPs are being increasingly bioinformatically-found in animal genomes, such as chelicerates, which elicit RNAi without RdRPs, suggesting alternative functions. I present results from microscopic analyses and cell culture-based experiments suggesting that *Ae. albopictus* RdRP-like nrVEs are regulated differentially in response to viral infections and negatively affect the replication of the insect specific-flavivirus cell fusing agent virus.

OC 2.2: Wolbachia strain wAu efficiently blocks arbovirus transmission in *Aedes albopictus*

Maria Vittoria Mancini, MRC-University of Glasgow Centre for Virus Research, UK

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The global incidence of arboviral diseases transmitted by *Aedes* mosquitoes, including dengue, chikungunya, yellow fever, and Zika, has increased dramatically in recent decades. The release of *Aedes aegypti* carrying the maternally inherited symbiont *Wolbachia* as an intervention to control arboviruses is being trialled in several countries. However, these efforts are compromised in many endemic regions due to the co-localization of the secondary vector *Aedes albopictus*, the Asian tiger mosquito. *Ae. albopictus*

has an expanding global distribution following incursions into a number of new territories. To date, only the wMel and wPip strains of *Wolbachia* have been reported to be transferred into and characterized in this vector. A *Wolbachia* strain naturally infecting *Drosophila simulans*, wAu, was selected for transfer into a Malaysian *Ae. albopictus* line to create a novel triple-strain infection. The newly generated line showed self-compatibility, moderate fitness cost and complete resistance to Zika and dengue infections.

OC 2.3: Arbovirus - mosquito interactions: Zooming in on transcription

Pascal Miesen, Radboud University Medical Center, The Netherlands

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Aedes mosquitoes transmit important arthropod-borne (arbo) viruses, including dengue and chikungunya virus (CHIKV). Whereas infections with these viruses can cause severe disease in humans, mosquitoes are infected by arboviruses but are generally able to keep replication at a sub-pathological level. Yet, the molecular mechanisms that underlie virus control are poorly understood. Therefore, we aimed to identify novel mosquito host genes that suppress arboviruses replication in *Aedes* mosquitoes. In all kingdoms of life, RNA binding proteins (RBPs) are well-known to regulate immune signaling in response to virus infections and we deemed it likely that members of this family control virus infection in vector mosquitoes. We therefore performed a knockdown screen, in which we silenced the expression of more than 450 RBPs in *Aedes aegypti* Aag2 cells and then measured replication of Sindbis virus, a model arbovirus from the same family as CHIKV. Silencing of putative antiviral factors is expected to increase virus replication, which we indeed observed after knockdown of 15 genes. These genes included potent regulators of transcription including genes that are involved in RNA-polymerase stalling and an RNA helicase that acts in controlling transcription of glycolysis genes in CHIKV infected mosquito cells. Our data indicate that several unexplored antiviral pathways cooperate to keep virus replication in *Aedes* mosquitoes in check. I will present a summary of our unpublished findings.

OC 2.4: Comparative analysis of the global virome of *Aedes albopictus* and *Aedes aegypti* mosquitoes

Joao Marques, Universidade Federal de Minas Gerais, Brasil

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Aedes aegypti and *Aedes albopictus* are the major vectors for arthropod-borne viruses (arboviruses) such as dengue and Zika. Virologic surveillance of mosquitoes has long been an important strategy to help raise preparedness and prevent outbreaks. It is unclear whether viruses can be exchanged between sympatric species of vector mosquitoes, which would affect how we monitor arboviruses in nature. Thus, characterizing the virome in different sympatric species of vector mosquitoes worldwide is of special interest. Viruses circulating in mosquitoes include not only arboviruses that infect humans and livestock but also insect-specific viruses (ISVs). Although these viruses do not directly infect vertebrates, mounting evidence indicates that they affect vector competence. Here, we analyzed *Ae. aegypti* and *Ae. albopictus* mosquitoes from Africa,

Europe, Asia and South America and identified 12 different viruses circulating in the two species. Viruses showed species-specificity and geographic restriction. *Ae. aegypti* mosquitoes often carried a more abundant and diverse virome compared to *Ae. albopictus* from the same geographical location. Our study begins to shed light on complex interactions between the natural mosquito virome and mosquito vectors that may affect the transmission of arboviruses in the wild.

OC 2.5: Provenance and competence in *Aedes albopictus*

Anna Malacrida, University of Pavia, Italy

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Davide Carraretto¹, Yoann Madec², Ludvik M. Gomulski¹, Anna-Bella Failloux², Eleonora Flacio³, Giuliano Gasperi¹, Anna R Malacrida

¹Dept. Biology and Biotechnology, Univ. of Pavia, Italy; ²Department of Virology, Arboviruses and Insect Vectors Unit, Institut Pasteur, Paris, France; ³Dept. of Environment, Construction and Design, Univ. of Applied Sciences and Arts of Southern Switzerland, Bellinzona, Switzerland

Aedes albopictus, the Asian tiger mosquito, is a case study of a successful human mediated global invasion. From a human health perspective, its invasion processes provide an evolutionary example of host-pathogen interaction. We found that the demographic history of *Ae. albopictus* populations is a consequence of a rapid complex pattern of diversification and divergence that influenced their competence for chikungunya virus (CHIKV). The demography of adventive populations was found associated with the CHIKV genotypes in a genotype-by-genotype interaction which impacted their vector competence. It follows that if populations from different geographical areas are related by their demographic history in terms of ancestry, they are likely to share similar levels of vector competence for a given CHIKV strain. On the other hand, this phenomenon generates variation in competence within regions such as the Mediterranean area where adventive populations are derived from admixture of different lineages. Here, having high or low level of ancestry in the different lineages impacts the population ability to be infected by CHIKV, the viral body titer and the degree of viral transmission. Moreover, within this region the populations are heterogeneous for dengue virus (DENV) which again is associated with their degree of ancestry.

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Session 3: Development, Physiology, ecology, Behaviour of *Aedes albopictus*

Chairpersons:

Isabelle Kramer, Goethe University Frankfurt, Germany;

Christophe Paupy, IRD Montpellier, France

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OC 3.1: The ecophysiological plasticity of invasive *Aedes* mosquito species - a comparative study of global, South Asian and European populations

Isabelle Kramer, Goethe University Frankfurt, Germany

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Vector-borne diseases and their respective vectors such as *Aedes albopictus* and *Aedes aegypti* have expanded their distribution range to cooler regions. *Ae. aegypti* majorly expanded its distribution in subtropical/tropical regions and *Ae. albopictus* also to temperate regions. To better understand the expansion potential of the two invasive *Aedes* species to colder regions we determined their ecophysiological plasticity in response to low temperature by means of a systematic literature review, cold tolerance experiments and genomic footprinting of climate adaption to colder ecoregions of especially *Ae. aegypti*. Worldwide, *Ae. albopictus* eggs are more cold-resistant compared to *Ae. aegypti*. Along an altitudinal gradient in South Asia, the overwintering capacity of the Lowland populations is high in *Ae. aegypti* and the species currently seem to adapt to the colder climate at higher altitudes. In contrast, the Highland population in *Ae. albopictus* shows the highest cold tolerance, which may be related to a longer establishment in this region if compared to *Ae. aegypti*. However, in comparison to South Asia, a European *Ae. albopictus* population exhibit the highest overwintering capacity with a high seasonal dependency. Driven by ongoing adaptation processes and climate warming, the two vector species will likely further spread to colder regions.

OC 3.2: Invasion of *Ae. albopictus* in forests of Central Africa: zoom on the situation observed in the National Parc of La Lopé (Gabon)

Judicaël Obame-Nkoghe, University of Science and Technology of
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The hypothesis of an invasion of forest environments of *Ae. albopictus* implies it has the ability to colonize forest ecosystems, including access to potential breeding sites, or feeding on wild reservoirs of arboviruses. The aim of our study was to demonstrate the potential of *Ae. albopictus* of invading wild forest habitats, and determine its degree of penetration in forest compartments from anthropo-sylvatic edges. To do this, in the Lopé national park, Gabon, we deployed during 5 days three networks of 30 to 40 ovitraps in three forest sites close to human habitations, over distances varying from 0 to 175m from edges towards deepest parts of the forest blocks. We also carried out larval surveys in natural and artificial water collections in the village and within the sylvan part of the park. Our results showed that there is a continuum of colonization of the forest area, showing that *Ae. albopictus* has the ability to colonize the interior of forests. However, a modeling of the colonization dynamics showed that its level of colonization decreases progressively with the distance from the anthropo-sylvatic forest edges. Larval prospections revealed that in the sylvan areas of the park, *Ae. albopictus* is more likely to colonize forest groves and galleries, known as circulation hub of animal reservoirs, and where it may act as bridge vector of zoonotic viruses between those forest areas and anthropogenic compartments.

OC 3.3: The eco-bio-social drivers of the dengue vectors *Ae. albopictus* and *Ae. aegypti*: An altitudinal transect study in Nepal

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Most recently, Nepal faced a nationwide dengue outbreak with >10,000 cases from 56 districts, including high mountain areas. To better design integrative and community-based measures for the prevention and control of dengue vectors, we conducted an entomological investigation on the occurrence and distribution of dengue vectors and studied their social determinants along a climatic gradient in Nepal. We found discarded car tires to be the most frequent larval production sites of mosquitoes in Central Nepal with the most abundant species *Ae. aegypti* (32%; up to 1800 m above sea level) and *Ae.*

albopictus (27%; 2100 m above sea level). In general, the collected mosquitoes show a broad vector competence for medically and veterinary relevant diseases and dengue vector species were frequently collected nearby houses. In contrast, the knowledge, attitude, and practice of people regarding dengue fever and vector control are quite poor. We recommend a) a nation-wide collection of discarded car tires from private properties and streets and making holes in tires to avoid water collection, b) to strengthen community participation to combat mosquitoes in peoples' backyard, c) the development of vector control strategies and preparation of health system for dengue and chikungunya diagnostics and treatment in the Highland areas of Nepal, d) and make a preparedness plan for outbreak of yellow fever, zika, West Nile, Saint Louis encephalitis, Japanese Encephalitis, and Rift Valley.

OC 3.4: Mosquito sex and active mycobiota contribute to fructose metabolism in the Asian tiger mosquito *Aedes albopictus*

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Plant floral nectars contain natural sugars such as fructose, which are a primary energy resource for adult mosquitoes. Despite the importance of carbohydrates for mosquito metabolism, a limited knowledge is available about the pathways involved in sugar assimilation by mosquitoes and their associated microbiota. To this end we used ¹³C-metabolomic and stable isotope probing approaches coupled to high-throughput sequencing to reveal fructose-related mosquito metabolic pathways and the dynamics of the active gut microbiota following fructose ingestion. Our results provide novel insights into mosquito carbohydrate metabolism and demonstrate that dietary fructose as it relates to mosquito sex is an important determinant of mosquito metabolism: our results also further highlight the key role of active mycobiota interactions in regulating the process of fructose assimilation in mosquitoes.

OC 3.5: A salivary factor binds a labrum cuticular protein and controls biting in mosquitoes

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The mosquito saliva is a cocktail of antihemostatic and immunomodulatory factors that shape the vector-host and vector-pathogen interactions. Here we show that a Labrum-Interacting Protein from the *Aedes* saliva (LIPS) can stimulate probing movements through direct interactions with the tip of the mosquito labrum, thus acting as secondary messenger in an unprecedented saliva-mediated feedback mechanism controlling the mosquito feeding on vertebrate skin. Silencing LIPS gene expression results in mosquitoes not capable of efficient probing prior to bloodfeeding. The LIPS function is reflected in its molecular structure, showing a new all-helical protein fold characterized by two domains, reminiscent of that found in extracellular signaling molecules. The N-terminal domain of LIPS interacts with a cuticular protein located at the tip of the mosquito labrum. Upon interaction, the morphology of the labral cuticle changes and this modification is most likely sensed by proprioceptive neurons. Our study identifies additional role of mosquito saliva in regulating the insect feeding that is highly relevant for the development of novel control strategies, and underlines that the external cuticle is a possible site of key molecular interactions affecting the insect biology and its vector competence.

Session 4: surveillance (distribution, resistance) of *Aedes albopictus*

Chairpersons:

Annelise Tran, CIRAD, France

Francis schaffner, Consultant, Switzerland

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Keynote Address 3: Using Precision guided sterile insect technique (SIT) to suppress mosquito populations

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Mosquitoes are considered to be the world's deadliest animals. They can transmit numerous pathogens such as Malaria and Dengue fever wreaking havoc on humanity. New technologies are needed to combat mosquitoes and the pathogens they transmit. Here I will describe a new CRISPR based technology termed precision guided sterile insect technique (pgSIT) which can safely be used to suppress and eliminate mosquitoes.

OC 4.1: ARBOCARTO: an operational spatial modeling tool to predict *Aedes albopictus* dynamics and the impact of vector control interventions

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Aedes albopictus and *Aedes aegypti* mosquitoes have a worldwide distribution and, as main vectors of dengue, chikungunya and Zika viruses, constitute a threat for public health both in tropical and temperate regions. To better target surveillance and control of *Aedes*-borne diseases, there is a need for tools with the capacity to predict the spatially distributed dynamics of mosquito vectors at a local scale. In addition, to be used by public health authorities and vector control services, such tools need easy-to-use interfaces

allowing a customization by the user according to the geographical and entomological contexts.

‘ARBOCARTO’ is a spatial modeling tool based on a generic mosquito life cycle-based model, driven by meteorological variables (temperature and rainfall). Its implementation considers the landscape context described from very high spatial imagery and/or ancillary data provided by the user, and the different functionalities allow the user to test different scenarios, such as the impact on mosquito dynamics of prevention measures (e.g., reduction of the number of breeding sites) or control actions (e.g., pulverization of insecticides). We present its application in various geographical contexts.

In highly diverse environments and latitudes, the comparison between the model outputs and observed entomological data demonstrated the ability of ‘ARBOCARTO’ tool to provide valuable complementary information to existing entomological surveillance programs. Thanks to its user-friendly interface, ‘ARBOCARTO’ could be adopted by a broad community of managers involved in vector control.

OC 4.2: Citizen-based surveillance of invasive mosquitoes within multi-sourced modelling

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Started in 2014 with the purpose of tracking the dispersal of *Aedes albopictus* in Spain, the citizen science project Mosquito Alert is now a near-real-time system aimed at the surveillance of four invasive *Aedes* species and of *Culex pipiens*, via citizen contributions through cellphones. The Mosquito Alert app is a data collection and communication system that allows to send adult mosquito pictures, to report breeding sites and biting activity worldwide, with an option to send actual specimens to reference labs. Images are validated by experts, who can also send feedback to participants, and then released to a webmap. To date, more than 211,000 people have registered the app, and the system has received 81,642 reports from 32,932 participants in 168 countries. Mosquito Alert is collaborating with national European Public Health agencies at different territorial scales across Europe by adding citizen science to their existing surveillance systems as a highly scalable, cost-effective, socially empowered and practically unlimited data source. Our scientific goal is to build epidemiologically-relevant, multi-sourced and data-driven models using quantitative approaches based on reliable estimates of mosquito abundance and activity (human-mosquito encounters) obtained from these novel digital data, in combination with traditional surveillance data sources, and sociological and environmental factors.

OC 4.3: Continental-scale surveillance of *Aedes albopictus*: standardisation for moving from distribution to abundance

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While *Aedes albopictus* has invaded most of southern Europe, it remains important to survey the species for its abundance, seasonality, and further spread or introduction. Through the VectorNet project, ECDC and EFSA are jointly supporting surveillance and data sharing for this species and other

arthropod vectors, at a continental scale. VectorNet maintains a vector distribution database filled via de collaboration of entomologists and analysis of publications for Europe and neighbouring countries and provides these data on request and through the publication of online six-month frequency updated presence-absence distribution maps. The 13-year series of updated distribution maps show the introduction, establishment and spread of the species, as a basic risk assessment for mosquito-borne diseases. Further, a map of the surveillance effort is produced. To improve the risk assessment, VectorNet has prioritised the analysis of data on vector abundance and seasonality. An attempt to produce a modelled continental abundance map for *Aedes albopictus* based on VectorNet data and complemented by additional data series was performed. The output maps showed clear limitation due to the heterogeneity of the training data, both in terms of methods and seasonality of the sampling, and, largely speaking, did not cover the likely extent of the vector species' range. In response, data reporting procedures for both field survey and literature extraction have been thoroughly revised, with much tighter and more comprehensive definitions of the sample effort metrics that need to be recorded to feed abundance models. This aligns with the aim for harmonisation of mosquito surveillance by the *Aedes* invasive mosquito (AIM) COST action and in particular the AIMSurg protocol implemented in 2020 and 2021. Longitudinal data collected by a harmonised field protocols and embracing the peak of activity of invasive mosquito species in 23 countries have already been gathered. The combination of these initiatives may improve the reliability of *Ae. albopictus* abundance and seasonality maps.

OC 4.4: TigeRisk: Evaluating the risk of insecticide resistance in the tiger mosquito: A predictive approach combining field surveillance, experimental selection and molecular markers

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By its key role in the transmission of arboviral diseases, the tiger mosquito *Aedes albopictus* affects public health worldwide. Though alternative arbovirus control strategies are being developed, targeting adults with pyrethroid insecticides (PYR) remains a common strategy to limit the risk of transmission. However as for other mosquito species, the tiger mosquito can adapt to insecticides by developing resistance mechanisms. PYR resistance is widespread in *Aedes aegypti* and has been shown to reduce the efficacy of insecticides-based control interventions in several tropical regions. Resistance has recently emerged and is currently spreading in *Ae. albopictus*. The rise of PYR resistance in *Ae. albopictus* represents a public health concern and managing resistance across territories is of key importance until novel control tools are widely implemented. However, this requires better understanding resistance mechanisms and improving their early detection in natural populations. In this context, the TigRisk project aims at anticipating the risk of PYR resistance in this mosquito species by combining field surveillance, experimental evolution and the development of new molecular tools for improving the tracking of resistance. The project includes mosquito populations from metropolitan France, southern Europe, the Indian Ocean, Africa and South East Asia. The project includes the following tasks:

- 1) follow PYR resistance levels at a regional scale
- 2) Use experimental evolution on various lines to characterise resistance mechanisms
- 3) Identify novel PYR resistance markers through combined genomic approaches
- 4) Design novel molecular assays to track resistance
- 5) Monitor the spatiotemporal dynamics of resistance alleles in the field and evaluate their usefulness for predicting resistance.

In this frame, this presentation provides an overview of the project and summarises the main results obtained by the consortium and its collaborators by spring 2022.

Session 5: *Ae. albopictus* control tool development and field evaluation

Chairpersons:

Alessandra della Torre, University La Sapienza, Italy

Charles Wondji, CRID, Yaounde, Cameroon

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Keynote address 4: *Aedes albopictus* insecticide Resistance

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Dengue control and prevention rely on insecticide-based interventions. However, insecticide resistance in dengue vectors such as *Aedes albopictus*, threatens the continued effectiveness of these tools. The design and implementation of resistance management strategies requires a good understanding of patterns of resistance to main insecticide classes as well as the underlying molecular basis of the resistance. Here, we present an overview of resistance patterns in *Ae. Albopictus*. Transcriptomic-based approach was used to detect the major genes driving metabolic resistance. Among these candidate genes, the Cytochrome P450 CYP6P12 was shown to be highly associated with pyrethroid resistance. Functional analyses further supported that CYP6P12 contributes to pyrethroid resistance in *Ae. albopictus* as transgenic expression of CYP6P12 in *Drosophila* was sufficient to confer pyrethroid resistance in these flies. Target site resistance was also investigated revealing the absence of the F1534C resistance allele in populations of this species suggesting that addition of the synergist PBO to pyrethroids could help overcome resistance in field populations of *Ae. albopictus*.

OC 5.1: Lethal and nonlethal effects of insecticides in *Aedes albopictus*

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Presentation of toxic sugar baits (TSB) to the *Aedes* mosquito vectors of dengue viruses (DENV) provides a form of vector control that takes advantage of sugar feeding behavior of adults. However, studies on the effect of ingestion of toxins in TSB ingestion on vector competence and vectorial capacity for viruses are lacking. This study evaluated vector competence for DENV-1 of *Aedes albopictus* at days 7 and 14 days post-ingestion of TSB formulated with spinosad as an oral toxin. Our results and others were modeled to estimate effects on *Ae. albopictus* vectorial capacity for DENV. Ingestion of TSB caused reduction of survival of females, but increased mosquito susceptibility to DENV infection, dissemination, and transmission. However, DENV titers in mosquito tissues were not affected. Despite the increase in vector competence, the reduction in survival led to a lower predicted vectorial capacity. The findings of this study highlight the importance of evaluating the net impact of TSB ingestion on epidemiological parameters of vectorial capacity in the context of vector control efforts to reduce risk of transmission of vector-borne viruses.

OC 5.2: SIT and IIT to tackle *Aedes* invasive mosquitoes: conjugating efficiency and economic sustainability

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Our commitment in research for improving the genetic control of the Asian tiger mosquito (*Aedes albopictus*) has gradually grown over the twenty years

of collaboration with the CAA (Center for Agriculture and the Environment) and joining network projects coordinated by the International Atomic Energy Agency, aimed at evaluating the added values of Wolbachia exploitation in the application of the SIT (Sterile Insect Technique). Thanks to these experiences we have increasingly understood the role of the Wolbachia endosymbiont bacterium both as a natural inducer of male "reproductive incompatibility" and as a blocker of arbovirus replication. In this presentation we report the progresses of SIT, in terms of biosafety, efficiency and sustainability, deriving from its evolution in IIT (Incompatible Insect Technique) that exploits the use of the Wolbachia bacterium as a source of male sterilization.

OC 5.3: New developments in the use of the Sterile Insect Technique against *Aedes albopictus* in Europe

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Mosquito-borne diseases represent a major threat to human kind. Dengue is a particular treat and WHO indicates the urgent need for alternative mosquito control methods, including the Sterile Insect Technique (SIT), which has progressed rapidly in recent years. The SIT package against mosquitoes is currently under development and huge progress was made in all components including mass-rearing of larvae and adults, sex-sorting of pupae, handling, transport, irradiation and aerial release of the sterile males. Testing is ongoing in the field following a phased conditional approach (PCA) has also been developed for the validation and potential large-scale deployment of the SIT against mosquitoes, as a component of an area-wide-integrated vector management strategy, based on the experience gained and concepts used to manage plant and livestock pests. In such a process, support or advancement to the next phase is conditional on the completion of all (or most) activities in the previous phase, and the scope, expense, and commitment increase along the process. In Europe, several countries initiated field trials against *Aedes albopictus*. Albania, Croatia, France (mainland), Montenegro and Portugal are

in phase 2 (baseline data collection) and three of them initiated mark-release-recapture experiments using irradiated males. Germany, Greece, Italy, Reunion island, Spain have started small-scale field trials (phase 3). We will review the progress of these countries along the PCA, showing that SIT was able to suppress target mosquito populations in various settings and present the perspectives in Europe, including through the IAEA technical cooperation project RER5026 “Enhancing the Capacity to Integrate Sterile Insect Technique in the Effective Management of Invasive *Aedes* Mosquitoes”.

OC 5.4: Pilot control trial by releases of sterile males against *Aedes albopictus* in Reunion Island

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The use of genetic vector control methods through the suppression of natural vector populations has recently emerged as an exciting and effective complement with the potential to improve the prevention of *Aedes*-borne diseases. Since 2009, the French National Research Institute for Sustainable Development (IRD) and scientific partners have been operating a SIT feasibility program for the suppression of urban populations of *Aedes albopictus* in Reunion Island. Our strategy relies for the most part on the mass-production and release of X-ray irradiated male mosquitoes to reducing the reproductive capacity of wild populations. This talk will provide a look at specific R&D processes, step-by-step, in enabling an effective pilot testing of SIT along with recent advancements.

OC 5.5: Autodissemination method against *Aedes albopictus* in a field experiment

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Aedes albopictus, since its installation in metropolitan France, represents an important source of nuisance in sectors where marsh mosquitoes have been

controlled for more than 60 years, it is also, and as episodes of autochthonous transmissions prove it every year, a vector of arboviruses of major health importance. Even if an emergency response plan exist with biocides, preventive actions have to be implemented to reduce the vector densities in urbanized areas. Community involvement is the most used strategy, whereas it's results are often disappointing. A method of self-dissemination (autodissemination) of larvicide was tested in a configuration representative and compatible with operational conditions near Montpellier. A decrease in adult densities was observed (up to 50/60% depending on the area), as well as an effective contamination of the breeding sites. These results are discussed from an operational control perspective.

OC 5.6: Insecticide resistance and population genetic analysis of *Aedes albopictus* populations in mainland Portugal

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The Asian tiger mosquito *Aedes albopictus* is a major threat to public health due to its ability for dengue, chikungunya and Zika virus transmission. This mosquito species has rapidly spread from Asia and is now considered one of the most invasive species worldwide. In Europe, the invasion started in Albania in 1979, having spread to 26 countries, including the Iberian Peninsula. During 2017, new detections of *Ae. albopictus* populations were recorded in Portuguese locations such as Penafiel (North) and Vilamoura (South). Therefore, this study aims to assess the genetic diversity and structure of *Ae. albopictus* using microsatellite markers and to assess insecticide susceptibility and presence of kdr mutations in both mosquito populations. *Aedes albopictus* larvae were sampled mainly from storm drains and reared in the insectary to produce an F1 generation to be used in susceptibility bioassays. Adult mosquitoes were sampled by landing collections and kept for genetic analyses. Six insecticides were tested using WHO assays and discriminant doses: Permethrin (0.25%), Deltamethrin (0.03%), Cyfluthrin (0.15%), Fenitrothion (1%), Bendiocarb (1%) and DDT (4%). Results showed susceptibility to pyrethroid insecticides (mortality >99%) but suspicion of reduced susceptibility to non-pyrethroid insecticides. Genomic DNA was extracted using the NZY tissue gDNA isolation kit (NZYtech,

Portugal). Genotyping of commonly present *kdr* mutations in this species revealed the absence of these mutations in Portuguese populations. Microsatellite markers were genotyped using previously published protocols and sent to Yale DNA Analysis Facility at Yale University for fragment analysis. Although our data indicates that in Algarve the mosquito might have arrived very recently, the diversity and genetic composition of the mosquito population in Penafiel indicates that in that location we have a well-established population. The fact that the mosquitoes were collected in a tire company might be related with possible multiple introductions in that site. Tires are imported from many suppliers including European and Oriental countries throughout the year and passive transportation of eggs should be an issue. The detection of *Ae. albopictus* populations in Portugal poses an important health risk for the human population. Therefore, controlling the spread of *Ae. albopictus* is mandatory to prevent and reduce the incidence of these diseases.

**OC 5.7: A review of pyriproxyfen-based larvicides against
Ae. albopictus and an introduction to a new long lasting
formulation of pyriproxyfen for use in containers and storage
vessels**

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SumiLarvTM 0.5G contains the insect growth regulator pyriproxyfen which is a highly effective larvicide/pupacide that has demonstrated excellent control of a wide range of mosquito species including *Ae. Albopictus*, across various studies and geographical locations. A field trial conducted in Malaysia showed that SumiLarvTM 0.5G applied at a dose rate of 0.02 mg active ingredient/L gave 100% emergence inhibition against the local population of *Ae. albopictus* for at least 10 weeks, regardless of the type of container (earthen jars or plastic tubs) and how frequently water was replaced (daily or weekly). Recently, Sumitomo Chemical developed a unique long lasting pyriproxyfen based larvicide, SumiLarvTM 2MR. This is a polymer resin-based disc shaped matrix release (“MR”) formulation that is very easy to use and has shown

exceptionally long residual efficacy against various mosquito species, with more than 6-month residual efficacy in drinking water. It is specifically designed for use in water storage containers and is approved for use in drinking water. These unique characteristics makes SumiLarv™ 2MR an effective and operational cost saving control tool. Both SumiLarv™ 0.5G and SumiLarv™ 2MR are WHO prequalified products. This presentation will provide a literature review on the efficacy of pyriproxyfen-based larvicides against *Ae. albopictus* and will include data on SumiLarv™ 2MR. SumiLarv is a trade mark of Sumitomo Chemical Co. Ltd

OC 5.8: Insecticide resistance in *Aedes albopictus* in Central Africa and the Prospects for control management

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Aedes albopictus is competent for dengue, zika and chikungunya viruses worldwide including Central African area. This area is endemic for several arboviruses of medical interest, however, no massive epidemic was reported before its invasion during 2000s. This important epidemiological change of DENV, CHIKV and ZIKV after introduction of *Ae. albopictus* have been seen in Cameroon, Gabon and Republic of Congo where even co-infection cases (DENV/CHIKV) have been observed. Due to the absence of vaccines and effective treatment, studies on the susceptibility of this vector to insecticides as well as mechanisms involved have been conducted in Cameroon, Central African Republic, Republic of Congo and Gabon to prevent and control a possible emergence. Overall, *Ae. albopictus* shows susceptibility to Bti and temephos. However, data with adulticides are alarming for more insecticide classes. In addition, the resistance profile vary according to the population origin. Indeed, the absence of kdr mutations cannot explain the decreased sensitivity observed, on the other hand, the activity of certain enzymes involved in metabolism could explain differences in susceptibility between populations. Considering that vector resistance is dynamic, regular evaluation becomes imperative for epidemic management.

Session 6: Public Health perspectives

Chairpersons:

Eleonora Flacio, University of applied sciences of Southern Switzerland

Omar Akbari, UC San Diego, USA

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OC 6.1: The quest for relevant indicators, evidence of effectiveness and improvement of Vector Control in France from 2010 to 2021

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Aedes albopictus is responsible of 30 outbreaks of dengue, chikungunya, and Zika in Europe since 2007. Various indicators are used to evaluate these vectorial situations. However, some important data are still harsh to obtain because of missing tools or poorly known methods. We present the indicators currently used in vector surveillance and control in France, and propose improvements based on case studies among the 23 autochthonous transmission events that occurred in mainland France between 2010 and 2021. During outbreaks, operators monitor entomological endpoints to improve the understanding of the conditions enabling a vector system to generate an outbreak. The *Stegomyia* indices (House, Container and Breteau indices) are the main quantitative indicators widely used for century for surveillance and control. However, the huge difference of mosquito productivity between containers is not considered by the traditional *Stegomyia* indices. Very productive containers can generate hotspots for vector-borne diseases transmission. As counting immature stages is time-consuming, estimation of productivity by visual comparison with Abacus method is very appropriate in the field. This method, already used for wetland mosquitoes, is reliable for container breeding mosquitoes. Nevertheless, *Stegomyia* indices are sometimes inadequate in temperate area because of diapause, with presence of adult vectors without larvAe. Risk indicator like adult abundance estimation by traps is difficult to implement in the field in an operational and rapid manner, considering that adulticidal treatments are performed as soon as possible in case of autochthonous transmission events. The use of biomarkers (antibodies) of human or animal exposure to vector bites could provide a view of the evolution of host-vector contact before and after treatments, and also allow an assessment of the effectiveness of vector control interventions. Finally, we will discuss the remaining challenges in evaluating the efficacy of newly applied procedures, such as mass trapping in areas where adulticide treatments are prohibited (streambank...).

**OC 6.2: Innovative biomarker of human exposure to *Aedes* bites:
from concept to applications to the assessment of the risk of
arbovirus transmission and vector control efficacy**

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The current emergence of arboviral diseases worldwide, the risk of their emergence in Europe and the frequent epidemics in French overseas departments, underline the needs to develop new monitoring and control tools. The study of human-*Aedes* immune relationships allow to define indicators of the risk of transmission and of the efficacy of vector control. One innovative approach is to develop biomarkers as quantitative and individual immuno-epidemiological tools to estimate the human exposure level to *Aedes* bites. After validation of the “concept” based on the measurement of human antibody response to *Aedes* whole saliva, we showed that only one salivary peptide (Nterm-34kDa) presented all criteria as biomarker of *Aedes* exposure and especially of *Ae. albopictus*. In mainland France, the assessment of specific IgG/IgM responses highlighted the heterogeneity of exposure to *Ae. albopictus* bites between regions and between the departments within colonized regions. In Reunion Island, IgG response to this salivary peptide was a short-time indicator for evaluating the efficacy of vector control interventions. The final step is to develop biomarker by rapid diagnostic test for its use at the operational level to survey the transmission risk and to evaluate the effectiveness of implemented vector control strategies.

**OC 6.3: Mass trapping and larval source management for the
elimination of *Aedes albopictus* from small tropical islands**

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Globally, environmental impact and insecticide resistance have forced pest control organizations to adopt eco-friendly and insecticide-free alternatives

to reduce the risk of mosquito-borne diseases. We report for the first time that, within a year, the combination of odour-baited mosquito traps and larval source management virtually eliminated high density populations of the Asian tiger mosquito *Aedes albopictus* (peak suppression 93,0% (95% CI 91,7-94,4)) and the Southern house mosquito *Culex quinquefasciatus* (peak suppression 98,3% (95% CI 97,0-99,5)) from a small Maldivian island. This eliminates the risk of mosquito-borne disease for local communities and safeguards tourism, which constitutes an essential economic resource. Furthermore, terminating intense insecticide use benefits human and environmental health and boosts insect biodiversity in fragile island ecosystems. Mass trapping therefore poses a convincing alternative to mosquito control based on insecticides and reaches impact levels similar to modern genetic control strategies.

OC 6.4: *Aedes albopictus* in France: Lessons learned and future challenges for surveillance and prevention, the French experience

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Aedes albopictus is continuously expanding in France since its first detection in 2004. A multidisciplinary plan for surveillance and control of viruses transmitted by *A. albopictus*, set up in 2006, allowed the detection and control of 23 transmission events affecting 1 to 17 cases. There were 19 events of dengue transmission (48 cases) and 3 of chikungunya transmission (31 cases). All chikungunya transmission events were caused by the ECSA lineage. The only episode of Zika transmitted by *Ae. albopictus*, detected in 2019, affected 3 cases and was due to the Asian genotype of the virus. All but one transmission events took place in areas and periods with high vector density. All cases occurred in 300m radius areas, mainly in peri-urban and residential areas. Main drivers of local transmission were a long delay or absence of control measures implemented around imported cases, the presence of wooded areas and the accumulation of heat during the season. The growing threat of arbovirus transmission, in the context of global change, represents numerous challenges for surveillance and control. We need to ensure the sustainability and efficiency of the system, to maintain and develop the capacity for rapid detection and risk assessment of new arboviral threats and

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to develop efficient control measures that also address the safety of substances of human origin (SOHO).

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Session 7: Public health concerns, management of control, market point of view

Chairpersons:

Carla Sousa, IHMT, Portugal

Guiliano Gasperi, Pavia University, Italy

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Keynote address 5: AIM-COST Action: results, lessons learned and future perspectives

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The Aedes Invasive Mosquitoes AIM-COST Action (CA17018) started in 2018 with the overarching objectives to develop a Pan-European network of scientists, public health officers and stakeholders involved in surveillance, study and control of Aedes Invasive Mosquitoes and to increase preparedness and capacity in detecting new invasive species and in reducing of the risk of arbovirus transmission in Europe. Currently AIM-COST includes members from 29 EU/neighbouring countries. One of the main specific objectives of the Action is to develop best practice guidelines/protocols ensuring consistency across countries and to standardise and streamline entomological and spatial analysis in order to promote enhanced risk assessments needed for reliable targeting and planning. The presentation will focus on the results obtained and on the lessons learned in the last years from the implementation of the first the first-ever Pan-European surveillance effort (AIMSurv 2020 and 2021) as well as from the implementation of citizen-science based monitoring activities (Mosquito Alert). Moreover, implementation and lesson learned from the comparison of different AIM control interventions within a Training School carried out in Valencia (2021) will be presented. Future activities and perspectives will also be discussed.

OC 7.1: The WHO response against *Aedes albopictus* through the WHO Global Vector Control Response (GVCR), the WHO Global Arboviruses Initiative (GAI) and the TDR/WHO supported activities

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VBDs such as malaria, dengue, Zika, chikungunya, yellow fever and others account for 17% of the total worldwide morbidity from infectious diseases – causing more than one million deaths per year, with few new drugs or strategies to combat these emerging infectious pathogens. The second most important vector for most of the arboviral diseases is the mosquito *Aedes albopictus*, originating from Asia but now expanding and invasive worldwide. The incidence of arboviral diseases has grown dramatically in recent decades due to several factors and global changes and has prompted WHO to state the urgent need for alternative vector control methods in its Global Vector Control Response (GVCR) 2017–2030, which was approved at the World Health Assembly in 2017 by more than 190 Member States. Another newly launched WHO Global Arbovirus Initiative is taking a global approach to surveillance, prevention and control of the arboviral diseases. Both GVCR and GAI will be presented as well as some supporting activities under our TDR Special Programme, such as the assessment of the countries capacities to prevent and control arboviral diseases and *Ae. albopictus* in the Caribbean and in Africa, the initiation of an international network to exchange on insecticide resistance, the support to an Innovative tool for Aedes eggs identification, the development of a tool for strengthening capacity building in medical entomology with the Global vector Hub and the testing of new vector control technology such as the Sterile Insect Technique. WHO commitment is very strong to provide to the countries the required support to better prevent and control the transmission of vector-borne diseases, including the control of major vectors such as *Ae. albopictus*.

OC 7.2: INOVEC: A Research & Innovation Partnership for enhancing the surveillance and control of mosquito vectors of emerging arboviruses

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Over the past 10 years, arboviral diseases, such as dengue, Zika, chikungunya and yellow fever, have (re)emerged with increasing prevalence and severity. Although these arboviral diseases are more prevalent in tropical countries, increasing numbers of autochthonous cases are being reported from European countries; hence raising concerns about the potential for the establishment of these pathogens in temperate regions. New, affordable, scalable and community-based vector control measures are urgently needed to prevent the introduction, spread and establishment of Aedes-borne diseases in Europe and beyond. The INOVEC consortium proposes to build a large pan-European, cross-sectoral and multidisciplinary network to develop, optimise and promote integrated approaches and innovative tools for the surveillance and control of mosquito vectors of emerging arboviruses. INOVEC gathers 21 academic and non-academic institutions specialized in vector biology, social sciences and product development to stimulate basic and applied research, strengthen capacities, promote career development and facilitate knowledge and technology transfer to countries at increasing risk of arboviral diseases. The final scope is to contribute to international efforts to improve global health and human well-being by reducing the burden of vector borne diseases.

OC 7.3: Bringing SIT to market in France: towards a more efficient control of the tiger mosquito

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New tools are needed for efficient control of tiger mosquito populations. The use of the sterile insect technique (SIT) as part of integrated strategies has

proven its efficiency in controlling this mosquito's populations in several countries. Our objective is to translate the technological developments and know-how developed by researchers into a viable commercial solution. Interest is high in municipalities and private companies in charge of guaranteeing low risk of pathogen transmission and/or biting nuisance. With its cooperative approach our company aims at securing an innovative and sustainable model for commercial SIT in France.

OC 7.4: Vectrap: Applicability of mass trapping strategy against *Aedes albopictus* and *Aedes aegypti*

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Aedes aegypti and *Ae. albopictus* are among the most important public health issues due to their ability to transmit human arboviruses, as well as a major source of nuisance. As the systematic use of insecticide against adults or larvae is an inappropriate strategy, the only accepted method of reducing the abundance of these vectors but also harming mosquitoes is through social mobilisation. Research and evaluation of new complementary methods without environmental and health effects remain a priority. For a few years, mass trapping is studied as a complementary or alternative control strategy. The objective of Vectrap project (PNR-EST-ANSES n°2020/1/114, 2020-2024) is to demonstrate the effectiveness, feasibility and acceptability of a new control strategy based on massive trapping against both species using 2 types of traps - catching gravid females and females in host research - and on citizen mobilisation. The project is conducted in 3 different regions (2 in metropolitan France, 1 in Martinique Island), with 8 sites for each location. Effectiveness is assessed in terms of reducing the number of mosquitoes, with the help of weekly or bi-monthly captures, and swap in behaviour and perceptions of the population based on psychometric studies (questionnaire surveys). The ultimate goal of the project, planned for 3 years, is to demonstrate the effectiveness of this mass trapping method in order to provide municipalities wishing to control these species with an acceptable and cost-effective strategy.

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Session 8: *Aedes albopictus* control in Europe: what is missing? Learning from other territories

Chairpersons:

Florence Fouque, WHO, Switzerland

Roger Eritja, CREA, Universitat Autònoma de Barcelona, Spain

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OC 8.1: Wanted: Cost-effectiveness of *Aedes albopictus* control strategies

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There is a dearth of evidence on cost-effectiveness of alternative *Aedes albopictus* control strategies/tools needed for effective planning of control. Randomized controlled trials (RCTs) with *Ae. albopictus*-transmitted arboviral diseases as epidemiological outcomes are impractical in non-endemic settings such as in Europe. This talk discusses modelling approaches and findings from a literature review on the correlation between epidemiological and entomological outcomes in (dengue, leishmaniasis and malaria) vector control intervention studies, and presents three small RCTs with entomological outcomes conducted in 2018 and 2019 in Albania, Italy and Spain, respectively.

OC 8.2: *Aedes albopictus* surveillance and control system (2000-2022): strategies and results in Switzerland

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Given the rapid spread of *Aedes albopictus* in Italy since the 1990s, in Canton Ticino (southern Switzerland) it was decided to start a surveillance system in 2000. Since the first tiger mosquito was found on Swiss soil in 2003, the surveillance system has constantly evolved, adopting different strategies according to the expansion of the insect, always with the aim of containing densities. Although it is impossible to limit the expansion of the tiger mosquito, the set of control measures adopted in Canton Ticino has been effective in containing its density, thus reducing both the nuisance for citizens and the risk of disease transmission. In recent years, the Ticino strategy has also been adopted in other Swiss regions under the supervision of the Swiss Mosquito Network supported by the Federal Office for the Environment.

OC 8.3: Monitoring data supporting larval control in urban areas of Emilia-Romagna, Italy

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In the Emilia-Romagna region, Northern Italy, the standardized *Aedes albopictus* monitoring by ovitraps during the summer season has been activated since 2008. A mean of about 2680 ovitraps, distributed in almost all Municipalities of regional plain area, operated in the period 2008- 2016, while since 2017 the number of ovitraps was reduced to 755, located in the ten main cities of Emilia-Romagna region. Ovitrap data are managed through a specific protocol and data validated by a quality control algorithm before being inserted into the database. Population density maps are produced monthly (June, July, August, September) by ovitraps data interpolation (Inverse Distance Weighted), allowing to recognize hotspots and assisting the larval control activities. The main larval control strategies implemented by the municipalities were the treatment of public catch basins, DtD (Door-to-Door) inspections and communication activities. All the acquired data, with reference to the product used for treating public catch basins, were recorded in the on-line database available at www.zanzaratigreonline.it. The summary time series data and density maps referred to the last five years (2017-2021) are presented in this communication.

OC 8.4: Tiger mosquito surveillance and control: the Spanish case

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Since 2008, the Spanish Ministry of Health has implemented a National Project for Entomological Surveillance, that includes the monitoring of mosquitoes in points of entry (Ports and Airports), and continuous tracking of invasive mosquito species, as well as the study of the possible emergence of insecticide resistance in populations. *Aedes albopictus* was recorded for the

first time in 2004 near Barcelona and since then it has gradually colonized Mediterranean territories, as well as inland and northern regions of the country. Faced with the geographic expansion of vector-borne diseases in 2016, the Ministry of Health published a National Plan for preparedness and response. Each territorial unit, called autonomous community, must coordinate and improve their own surveillance programs of tiger mosquito and adapt their plans against vector-borne diseases. However, in general the implementation of mosquito control plans, is the responsibility of local governments via the recruitment of private pest control companies, causing a great deal of inequality between regions, always dependent of the particular interest to limit the densities of mosquitoes in its territory. Past and current known situation, as well as different initiatives underway will be exposed.

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P1: Assembling De Novo *Aedes* genomes from Single Specimens

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Owed to their importance as vectors and the promise of genome sequences unlocking new methods of control, genomic resources for some *Aedes* and *Anopheles* species are among the best in the world. However, most of these genomes are based on combined pools of many individuals, in order to meet DNA input requirements. Recent advances in genome sequencing technology by Pacific Biosciences (PacBio) have made it possible to produce large quantities of highly accurate long reads (HiFi) from single, wild-caught individuals to generate genomes from individual mosquitoes. In response, numerous new genome assemblers have been written to specifically handle these new datasets. These assemblers have not been directly compared in mosquitoes and share a common problem in that they artefactually duplicate heterozygous regions of the genome. To solve this problem, other programs have been written to purge these duplicate sequences. Here, we use HiFi reads from *Aedes aegypti aegypti* and *Ae. aegypti formosus* to evaluate the performance of four HiFi assemblers—HiCanu, HifiASM, flye, and IPA—and two purging programs—purge_dups and purge_haplotigs. We evaluate performance by quantifying N50, assembly size, and BUSCO scores, and compare them to the *Ae. aegypti* reference genome. We find that no single combination of assembler and purging program outperforms other combinations on all metrics. However, numerous combinations produced assemblies comparable to published *Aedes* reference genomes. Further optimizations in assembler and purging settings may further improve assembly quality.

P2: A Genotyping-in-Thousands by sequencing (GT-seq) panel development and applications to support studies in molecular ecology in the tiger mosquito (*Aedes albopictus*) populations worldwide

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Estimating population structure, dispersal, invasion routes and history, and key demo-genetic parameters from population genetic data can help unravelling the success of the highly invasive tiger mosquito (*Aedes albopictus*). However, such endeavor is still limited by the lack of high-throughput and cost-effective genotyping assay capable of handling low-input and low-quality DNA. Relying on a de novo restriction site-associated DNA (RAD) sequencing assembly from published data representative of the worldwide genetic diversity of the *Aedes* mosquito populations, we designed and tested the ability for a multiplex amplicon sequencing approach to effectively genotype thousands of individuals using the GT-seq protocol and next-generation illumina sequencing platform. We identified 13,800 single nucleotide polymorphisms (SNPs) distributed on 433 distinct RAD-loci. We show that this GT-seq panel of SNPs is capable of closely reproducing the previously described worldwide population genetic structure and diversity. It also offers very polymorphic microhaplotypes for demo-genetic applications such as fingerprinting and close-kin-mark recapture approaches. Being applicable with low-input and degraded DNA, this GT-seq amplicon panel will allow the expansion of molecular ecology studies in *Aedes albopictus* while minimizing the potential risks of ascertainment bias.

P3: Cell Fusing Agent virus and *Aedes* spp. mosquitoes: infection kinetics and transmission mechanics

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In 1974 an insect specific virus (ISV) was discovered in *Aedes aegypti* cells and named Cell Fusing Agent Virus (CFAV). It was not until 2006 when the virus was seen in a mosquito for the first time and since then CFAV has been found in entomological surveys of *Aedes albopictus* and *Ae. aegypti* around the world. Recently, CFAV and other ISVs have been proposed as possible alternatives for biological control of arboviruses. Despite the increasing importance of CFAV, there is still a paucity of data regarding its mode of transmission in *Aedes* spp. mosquitoes. In this study, we analysed the kinetics of ovarian infection and the mode of transmission.

P4: Phenotypic and transcriptomic analyses of abnormal male sexual development in *Aedes aegypti* and *Ae. mascarensis* backcross progeny

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When male hybrids of *Aedes aegypti* females and *Aedes mascarensis* males are backcrossed to *A. aegypti* females, one part of the male backcross progeny consists of sterile intersexes. In this study, we characterized developmental abnormalities associated with intersex phenotypes in the *A. aegypti* / *A. mascarensis* 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 antenna, external genitalia, and reproductive organs consisting of both ovarian and testicular parts. We conducted RT-PCR using primers for Nix and splice variant specific primers for doublesex and fruitless. 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. These results suggest that the intersex phenotypes are caused by a genetic conflict between the *Ae. mascarensis* gene(s) in the M locus and the *Ae. aegypti* downstream targets of the sex determination pathway.

P5: Role of Toll, Imd, and Jak-STAT pathway in antiviral immunity in *Aedes albopictus*

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Aedes albopictus is a vector for transmission of important human pathogens such as Dengue virus and Chikungunya virus. Better understanding of the ability of *Aedes albopictus* to transmit arboviruses to humans (i.e. its vector competence) is critical to prevent and/or control future arbovirus outbreaks. Antiviral immunity of the mosquito is a key determinant of its vector competence, but is incompletely understood. It has become clear that mosquitoes use the RNA interference pathway to restrict arbovirus replication. Induced transcriptional responses mediated by the Toll, IMD and Jak-STAT pathway are also thought to control virus replication, but the antiviral role in mosquitoes, in particular in *Aedes albopictus*, is currently poorly understood. We are studying these pathways and their signalling components, exploring the transcriptional responses, and characterising the relevance of these pathways for antiviral immunity in *Aedes albopictus*. To this end, we have characterised the different splice variants of the Toll, Imd, and Jak-STAT transcription factors using RT-PCR and rapid amplification of cDNA ends (RACE) assays. We observed four Rel1a (Toll pathway), one Rel2 (Imd pathway), and three STAT splice variants, a pattern that is highly similar to the splice variants that are annotated in the *Aedes aegypti* genome. For STAT, an N-truncated splice variant was observed which is expected to be a negative regulator based on its protein domain composition. In addition, using heat-inactivated gram-positive and gram-negative bacteria as stimulants we defined a group of Toll/Imd-responsive genes in *Aedes albopictus* U4.4 cells. These tools will be used to test if different arboviruses and insect-specific-viruses activate the Toll and Imd pathways.

P6: Impact of environmental yeasts on the development of the tiger mosquito and assessment of their attractiveness

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The microbiota of the tiger mosquito (*Aedes albopictus*), mainly acquired from the environment, can influence many aspects of its biology such as its development, physiology or behavior. Unlike the bacterial fraction, the impact of yeasts on the biology of *Ae. albopictus* has been largely understudied, even though they represent up to 84% of the mycobiota. The main objective of this work was to determine whether environmental yeasts that promote the development of larvae attract gravid females and influence the selection of oviposition sites. Ten yeasts isolated from breeding sites were analyzed for their ability to restore the development of axenic larvAe. The results show that all yeasts restore the larval development but that the development time until the adult stage is variable. The presence of these different yeasts during larval development has a repercussion on certain life history traits at the adult stage since slower larval development has been associated with smaller and protein-rich adult individuals. Under our experimental conditions, yeasts showing the higher larval development rate were not associated with a preferential choice of gravid females in behavioral dual-choice experiments.

P7: Identification and characterization of unprecedented interactions between LIPS-2 and Cp19 at the *A. albopictus* labrum tip

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Mosquito saliva is a complex molecular cocktail enriched with several macromolecules that play critical roles in blood feeding. Among these, using

gene silencing approaches we identified proteins that are essential for proper control of the mouth probing movements inside the host skin preceding blood feeding. Based on our discoveries, we named these proteins Labrum Interacting Protein from Saliva (LIPS), and we focused on the LIPS-2 isoform. We found that LIPS-2 binds to the tip of the mosquito labrum and that these contacts induce morphological changes in the labrum, triggering the typical probing movements. We determined the crystal structure of this protein, revealing a novel, all-helical fold composed of two domains but lacking insights on its possible mechanism of action. We then opted for using yeast 2-hybrid assays to identify a candidate interactor of LIPS-2 within the labrum cuticle and found a candidate binder in Cuticular protein 19 (Cp19). Immunolocalization studies showed that Cp19 is embedded in the chitin of tip of the mouth's labrum and that its C-terminus is critical, but not sufficient for LIPS-2 interaction. Microscale thermophoresis (MST) experiments using recombinantly expressed, fluorescently labeled proteins provided a qualitative and quantitative validation of the LIPS-2:Cp19 interactions, also providing a preliminary characterization of the molecular surfaces involved in protein-protein interactions. Collectively, our results shed light on an unprecedented feedback mechanism critical for probing and subsequent blood feeding in *Aedes* mosquitoes. Future work will focus on structural and functional characterization of the LIPS-2/Cp19 contacts and drug discovery campaigns aimed at interfering with these processes, to establish innovative strategies to fight mosquitoes.

P8: A few weaknesses in the *Aedes albopictus* biology

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The worldwide success of *Aedes albopictus* is impressive.

It relies on number of biological features among which:

- the ability of egg stage to resist desiccation for months,
- the potential to colonize temperate areas thanks to diapausing eggs,
- the appetite of adults and aquatic stages for human environment,
- a large range of endo/exo-philic behaviours that favours entry/exit in/from house, car, boat, plane, which greatly increases the long distance dispersion (up to an intercontinental scale),

- a large range of antroponophilic behaviours which permits the exploitation of a variety of blood sources,
- a fitness advantage at larval stage (demonstrated at least against *Ae. aegypti*).

Briefly, any biological feature appears to be a strength with a noticeable exception: the dispersion of flying females is estimated at 100-150 m during her lifespan, indeed one of the lowest flying distance for adult mosquito; and it is a paradox that such an invasive species is composed of sedentary individuals!

This weakness suggests that elimination of this mosquito from an area is sustainable as soon as an efficient barrier is active around a 100-150 m perimeter.

P9: X-ray Tomography as a new tool to study the development of *Aedes albopictus* mouthparts

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The buccal apparatus of adult female mosquitoes is composed of the labium, a protective sheath retracted during the blood meal, and of a fascicle of sharp-pointed stylets, i.e., the labrum, the hypopharynx, a pair of maxillae and a pair of mandibles, which are inserted in the vertebrate host skin. The timeline of the formation of mosquito mouthparts, which occurs during pupal development, together with their reciprocal organisation, has not been already clarified, mainly because of challenging sample preparation for classical imaging analysis, including Transmission Electron Microscopy. Indeed, mouthparts are covered by cuticular layers, mostly composed of chitin, which is hard to embed and to section into resins. In this regard, X-ray Tomography was exploited to realise segmentations and 3d reconstructions of the buccal apparatus of 4th instar larva, male and female adult mosquitoes, and female pupae at different time points after pupation (0, 1, 2, 3, 4, 5, 6, 7, 8, 20, 30, 48 hours). Datasets were collected at the synchrotron facility

(Villigen, CH) and analysed in both the absorption based and edge-enhanced radiography, using Gridrec and Paganini reconstruction guidelines. This approach allowed to highlight similarities, as well as differences, in the organisation and development of mouthparts in close-related species (*Aedes albopictus* vs *Ae. aegypti*), as well as of species of different genera (*Anopheles* vs *Aedes*).

P10: *Aedes albopictus*: a "sleeper" vector of arboviruses in Madagascar

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Aedes albopictus, introduced to Madagascar from Indo-Malaise, was first reported in 1904 on the island. Historically, *Ae. albopictus* and *Ae. aegypti* were geographically separated with the former species being abundant in eastern and highland areas whilst *Ae. aegypti* in western and southern regions. However, data from 2019-2020 showed an unexpected sympatric distribution of both species in all five bioclimatic domains. Exophilic and endophilic populations of both species were also highlighted. Larval stages of both species were found colonizing a wide range of habitats and co-existing in bamboo, tree holes, leaf axils, rice fields, metallic and plastic recipients, gutters and cement water tanks. We then performed insectary experiments to assess the breeding habitat preference and larval interspecific competition of both species. The results showed a stable co-existence in most container types although *Ae. albopictus* larvae outcompeted *Ae. aegypti* ones in metallic containers. When screening for arboviruses, only Babanky and Rift Valley fever viruses were isolated from wild *Ae. albopictus* in Madagascar, although Dengue and Chikungunya viruses are widely circulating in the western Indian Ocean. These new insights on *Ae. albopictus* biology and range expansion open new questions about the origin and evolution of its adaptation to different environmental settings and its potential role in transmission of additional human arboviruses

P11: The “domestication” process of *Aedes albopictus*: lessons from *Aedes aegypti*

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The domestication of plants and animals has transformed the organisms involved but the entire human evolution and the ecosystems that occupies. The process is divided in the origin, the dispersal and the changes linked to domestication. Mostly studied in cattle and crops, the evolution of domestication is poorly known when non-human mediated selection is involved. Main mosquitoes' vectors were primarily attracted by human-altered habitats and their role in transmitting diseases appear to be a side effect. Before *Aedes albopictus* pullulated our cities, *Aedes aegypti* successfully travelled around the world. In their ancestral geographical areas, their remarkable ecological larvae plasticity may responsible to their human adaptation. While the role of climate conditions has been incriminated into the first human contact in *Ae. aegypti*, it is not clear in *Ae. albopictus*. Moreover, out-of-Africa populations of *Ae. aegypti* exhibit a high degree of anthropophily (preference for feeding on humans), while *Ae. albopictus* remains opportunistic through their actual range. Despite the immense progress understanding host preference and vector competence, in this perspective work, we tackle the key challenges that remain to understand the nascent of domestic populations of these two major arbovirus vectors, including genetics of domestication, reproduction, survival or morphological changes from their wild ancestors.

P12: Field Evidence of *Aedes albopictus* Population Regulation by a Gregarine Parasite

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Although parasites are by definition costly to their host, demonstrating that a parasite is regulating its host abundance in the field can be difficult. Here we present an example of a gregarine parasite, *Ascogregarina taiwanensis* Lien

and Levine (Apicomplexa: Lecudinidae), regulating its mosquito host, *Aedes albopictus* Skuse (Diptera: Culicidae), in Bermuda. We sampled larvae from container habitats over 2 yr, assessed parasite prevalence, and estimated host abundance from egg counts obtained in neighboring ovitraps. We regressed change in average egg count from 1 yr to the next on parasite prevalence and found a significant negative effect of parasite prevalence. We found no evidence of host density affecting parasite prevalence. Our results demonstrate that even for a parasite with moderate virulence, host regulation can occur in the field.

P13: Bacterial symbiosis in *Aedes albopictus*

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The microbiota associated with an Italian population of *Aedes albopictus* has been characterized showing a complex composition in different organs like gut, salivary glands and reproductive organs. We have mainly concentrated our attention to the acetic acid bacterium *Asaia* that is one of the best characterized mosquito symbionts. We show that phylogenetically distant lineages of *Asaia* experienced independent genome reductions, despite following a common pattern, characterized by the early loss of genes involved in genome stability. This result highlights the role of specific metabolic pathways in the symbiotic relationship between *Asaia* and the insect host. Finally, *Asaia* strains isolated from *Aedes albopictus* and other mosquitoes, possess a pyrethroid hydrolase gene. We propose that this gene from the symbiont contributed to initial pyrethroid resistance in insects harboring *Asaia*, also considering the widespread production of pyrethrins by several plants.

P14: Not only smell? The peculiar case of Obp47 of *Aedes albopictus*

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Odorant Binding Proteins (OBPs) in mosquitoes represent complex families of proteins, including members with diverse and unrelated functions, of which olfaction is only one. We have experimentally determined the first three-dimensional structure of a member of the C-plus class of OBPs in *Ae. albopictus* (AealbOBP47) expressed mainly in the antennae and in palpi, showing however peculiar differences between sexes in some body compartments. The AealbOBP47 crystal structure reveals a compact architecture, kept together by six disulfide bonds distributed throughout the polypeptide chain. Superposition of this structure with available orthologous structures of C-plus OBPs from *Anopheles gambiae* (AgamOBP47 and AgamOBP48; structure identity greater than 50%), showed that the major differences are present at their flexible N- and C- termini, kept together by a disulfide linkage and shaping a possible gating element involved in conformational regulation of the molecule. Similar to AgamOBP47, the AealbOBP47 surface is characterized by two crevices interconnected by a tight channel defined by the conformation adopted by N- and C- termini of the molecule. Different from what observed for AgamOBP48, AealbOBP47 is monomeric in solution, suggesting that either one of both crevices observed within its fold might be involved in ligand binding and/or in interactions with other macromolecular partners. Analysis of the amino acid network shaping both crevices highlights abundance of charged and polar side chains, suggesting that, unlike classical OBPs, AealbOBP47 could favorably interact with hydrophilic molecules. This was corroborated by the lack of bind to the classical hydrophobic probe 1-NPN. To assess the ability of AealbOBP47 to interact with odorant and candidate binders, we therefore established biophysical assays based on differential scanning fluorimetry. We tested a large pool of candidate odorant compounds, and unexpectedly found that none of these molecules interacts with this macromolecule in vitro. Combined with the unusual structural features of this protein, our findings raise question regarding the actual function of this protein in *Ae. albopictus*. More generally, this work contributes new insights on the mysteries of C-Plus OBPs, their molecular architectures and functional implications.

P15: Human-aided dispersal facilitate parasitism escape in the most invasive mosquito species.

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Human aided invasion of alien species across the world sometimes lead to economic, health or environmental burdens. During invasion, species may encounter new environments and partially escape some ecological constrain that were present in their native range while they face new ones. The Asian tiger mosquito *Aedes albopictus* is one of the most emblematic invasive species that was introduced in 5 of the 6th continent in a short period of time due to international traid. In this study, we showed that *Ae. albopictus* populations are highly colonized by parasites. However, we also evidenced that (i) several genotypes colonizes mosquitoes and that (ii) recently introduced populations are poorly colonized by the parasite. Parasitism escape may favor the mosquito but reason for such pattern were still unclear. Several hypothesis were tested under controlled experiments. First, we showed that a decrease of local mosquito dispersion was unlikely since infected mosquitoes tend to fly more than uninfected relatives. Secondly, we assumed that founder effects may influence the parasite dynamics (i.e. sall populations after introduction). However, this was independent of the density of host individuals but rather likely to be due to reduced number of parasites released. Finally, we showed that egg desiccation, that is mandatory for human aided transportation across long distances, decreases the number of parasites that efficiently colonize the mosquitoes.

P16: Study on the viability of the *Aedes albopictus* eggs over a period of 2 years

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The purpose of our study was to evaluate the viability of the *Aedes albopictus* eggs combining different conditions. The eggs of *Aedes albopictus* were captured by ovitrap in the temperate and urbanized city of « La Garenne Colombes » (in the « Ile de France » Region) where the colonisation of *Aedes albopictus* is no longer to prove. To manage this study, we evaluate some eggs from ovitraps placed in the month of August 2019, when the peak of this mosquito's activity is the highest. The different conditions of diapause tested included the variation of the environment, temperature and lighting. Some eggs were placed in dry and in wet earth (mud) under laboratory conditions (12/12 photoperiod, 21-23°C temperature, 65-75% humidity). Other eggs in the dry state and in the mud were placed under the natural climatic conditions. After 8 months, 5 to 30% of the eggs under laboratory conditions have hatched and 19 to 39% of the eggs placed under natural conditions have hatched. On the other hand, no hatching was observed after one year and two years for eggs stored in the laboratory or under natural conditions.

P17: *Aedes albopictus* in hyper-urbanized areas: the case of Paris

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In metropolitan France, *Aedes albopictus* (tiger mosquito) has settled on the French Riviera since 2004. To limit the risk of importation and establishment of vector-borne diseases, the French Ministry of Health developed in 2006 a national program against the spread of chikungunya, dengue and Zika. In November 2018, the tiger mosquito (*Aedes albopictus*) was established and active in Paris, classified at level 1 of the national plan for control in metropolitan France. We present here the assessment of the entomological surveillance implemented in Paris for the 2019 season, with a focus on the urban specificity of Paris (diversity of potential lodgings, problem of entomological surveys in hyper-urbanized areas) and the implementation of prevention actions.

P18: Surveillance entomologique et lutte contre *Aedes albopictus* dans la région des Pays de la Loire

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L'implantation d'*Aedes albopictus* en Pays de la Loire est relativement récente. Après une première détection en 2014 dans le département de la Vendée, la colonisation a lentement évolué dans la région. Au 1er janvier 2022, 9 communes réparties dans 4 départements sont colonisées. La surveillance entomologique s'appuie sur deux grands moyens, la surveillance passive par signalements citoyens et la surveillance active par un réseau de 185 pièges pondoires. Les détections qui en résultent dans des zones non colonisées entraînent automatiquement la réalisation d'enquêtes entomologiques dans un rayon de 150 mètres autour de leur localisation. Ces interventions visent à localiser et à supprimer les gîtes larvaires et de repos du moustique tigre tout en sensibilisant les citoyens. Les déclarations de cas d'arboviroses engendrent également des enquêtes pour vérifier l'absence d'*Aedes albopictus* autour des différents points de passage du malade durant sa période de virémie. Les opérations de démoustication par traitements adulticides ne sont engagées dans la région que lorsqu'il existe un risque sanitaire de contamination.

P19: Spatial distribution and insecticide resistance profile *Aedes albopictus* compare of *Aedes aegypti* in Douala, the most important city of Cameroon

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Prevention and control of Aedes-borne viral diseases such as dengue rely on vector control, including the use of insecticides and reduction of larval sources. However, this is threatened by the emergence of insecticide

resistance. We assess the spatial distribution and the insecticide resistance profile of *A. aegypti* and *A. albopictus* in Douala city. Immature stages of *Aedes* were collected in August 2020 in eight neighbourhoods and reared to adult stage. Adult bioassays, and piperonyl butoxide (PBO) synergist assays were carried out according to WHO recommendations. Overall, *A. aegypti* is the predominant *Aedes* species but, analyses revealed that both *A. albopictus* and *A. aegypti* coexist in all the prospected neighbourhoods of Douala. However, *A. aegypti* is more prevalent in downtown neighbourhoods while *A. albopictus* is the predominant species in the suburbs. High level of resistance was observed to three pyrethroids tested in both *Aedes* species with low mortality rate in *A. albopictus*. These findings provide relevant information's which should be capitalised in the implementation of arbovirus vectors control strategies.

**P20: 2006-2021: assessment of 16 years of corsican colonisation
by *Aedes albopictus* (Aedinae - Culicidae)**

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In 2006, the discovery of the installation (probably in 2005) of *Aedes albopictus*, potential vector of arboviruses (Chikungunya, Dengue, Zika...) in Corsica led to the setting up of surveillance by ovitraps all over the island. This made it possible to follow its colonisation, to determine the activity period of the mosquito and to evaluate the seasonal dynamics of the populations. At the same time, door-to-door surveys were carried out punctually to estimate peoples' attitudes and knowledge about this mosquito, to understand the typology of breeding sites and to assess the density of tiger mosquito populations. For it, entomological indices such as house index, Breteau index, pupae index ... have been calculated. At the end of 2021, 98% of the municipalities were colonized. This monitoring has shown us that the entomological risk of disease transmission and the case import risk were the highest at the same time. More than 80% of the breeding sites were containers of less than 50 liters and even more than half could contain less than 10 liters. Large containers were still the most productive. All these studies carried out over the years told us that people know the tiger mosquito

better but the application of advice remains a problem because the concerned people are not necessarily aware of being mosquito breeders.

P21: Application of a new intelligent sensor for the remote monitoring of mosquitoes in the field

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Mosquito surveillance programs are needed to monitor mosquito populations, detect the presence of potential invasive species, identify arbovirus circulation, design control strategies, and implement quality control assessment. Traditional entomological surveillance methods are very time consuming and require expert entomologists. New insights in this field include the use of either acoustic or optic sensors trained with machine learning techniques to remotely classify adult mosquitoes based on their flight. The vast majority of these studies have been performed in laboratory conditions, reporting promising levels of accuracy for the classification of mosquito genus, specie or sex. However, there is a lack of studies which report results of the use of these intelligent sensors in the field, where there are several uncontrolled environmental variables that may affect the flight pattern of the mosquitoes and consequently, the classification process. In the current work, we present a novel optoelectronic sensor prototype attached to the entrance of a commercial adult mosquito trap which is capable of remotely classify mosquitoes in terms of genus and sex with high levels of accuracy (94-100%). The focus of this study is on the field trial of the sensor which took place during the summer of 2021 in the province of Barcelona, Spain. The results showed that there was a positive correlation between the data predicted by the sensor and the data found by the manual inspection of the traps. These encouraging results are a proof of fact that this technology could be incorporated to the routine of mosquito surveillance programs with the advantage of generating effortless real-time data.

P22: The progressive expansion of *Aedes albopictus* across the Alps

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Aedes albopictus is firmly established south of the Alps while its dispersal pattern across the Alps remain less consistently assessed, limiting a projection of future scenarios and impeding a more targeted mosquito surveillance. To identify the origin of *Ae. albopictus* and dispersal pathways across the Alps, we analysed data from > 4000 genome-wide single-nucleotide polymorphisms obtained through double digest restriction-site associated DNA sequencing, collecting specimens from Switzerland, Europe, Americas and Asian range. Additionally, we monitored the presence and frequency of invasive *Aedes* mosquitoes across the Alps over consecutive years deploying a sentinel trapping network consisting of oviposition and BG-Sentinel traps at potential points of entry across Switzerland. We detected a weak genetic structure and high levels of genetic admixture, supporting a scenario of rapid and human-aided dispersal along transportation routes. Surveillance data confirm passive dispersal of *Ae. albopictus* across the Alps, with the E35 as a key route of introduction. The recovery of a pair of full siblings in two consecutive years in Strasbourg, France, suggests the presence of overwintering populations north of the Alps. This finding together with the expansion patterns points to an increased risk of further northwards expansion and the need for increased surveillance of mosquito populations in northern Europe.

P23: From a long-distance threat to the invasion front: a review of the invasive *Aedes* mosquito species in Belgium between 2007 and 2020

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Invasive mosquito species and their associated mosquito-borne diseases are emerging in Europe. In Belgium, the first detection of *Aedes albopictus* occurred in 2000 and of *Aedes japonicus* in 2002. Between 2007 and 2020 52 points-of-entry (PoE) were monitored at least once for the presence of invasive mosquito species. In general, monitoring was performed between April and November using adult and oviposition traps, as well as by larval sampling. *Aedes albopictus* has been detected at ten PoE's, *Ae. japonicus* at three PoE's and *Aedes koreicus* at two PoE's. The latter two species have

established overwintering populations. In Belgium, the import through passive ground transport was first recorded in 2018 and its importance seems to grow. Belgium is currently at the invasion front of both *Ae. albopictus* and *Ae. japonicus* and *Ae. albopictus* is expected to become established in Belgium in the coming years. Hence increasing the likelihood of local arbovirus transmission. The implementation of a sustainable, structured and long-term Invasive mosquito species management programme, integrating active and passive entomological surveillance, vector control and public health surveillance is therefore pivotal.

P24: Wolbachia-based suppression of *Aedes albopictus*: results of a biennial field experiment in Rome

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Wolbachia IIT is a population suppression approach whereby male mosquitoes, infected with Wolbachia, are released into a wild population lacking that Wolbachia strain. Mating between Wolbachia-infected males and wild females results in nonviable offspring. ENEA has created an artificial *Aedes albopictus* line (ARwP) that has high penetrance of sterility in matings between Wolbachia-infected males and wild females (i100%) and ensures similar mating competitiveness between Wolbachia-infected and wild males. In June/July 2018 we started to test IIT in open field (2.5 ha) in Rome (Italy). About 4,500 and ~5,000 ARwP males/week/6 weeks, were released in 2018 and 2019 respectively. The incompatible to wild male ratio and the average egg fertility in treated and in control sites were assessed. The percentage of viable eggs during release was, on average, significantly lower in treated sites than in control sites. Approximately, 30% in 2018 and 22% in 2019 of females collected in the release spots showed 100% sterility even if inseminated. Results support the potential of IIT against mosquitoes. The exploitation of incompatible males as a suppression tool may be synergically combined with other control strategies to realize an integrated Vector Management of *Aedes* species.

P25: Molecular survey of the F1534C knockdown mutation in *Aedes albopictus* from Europe

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Aedes albopictus global expansion in the last four decades increased public health concerns on transmission of arboviruses since no vaccine or therapeutic approaches are available. Insecticide-based control methods against mosquito vector populations are the major control strategy, however intensive use of pyrethroids (PY) increases the risk of emergence of insecticide resistance. Mutations in voltage gated sodium channel gene (*vgsc*) are associated with knockdown resistance (*kdr*). We carried out a continent-wide survey of the F1534C mutation in *Ae. albopictus* populations across the European continent, considering a total of 1,629 field-caught specimens from 18 countries. Allele Specific Polymerase Chain Reaction (AS-PCR) technique was used to detect F1534C mutation, followed by the sequencing of a 10% subsample that included a 264 bp fragment containing position 1534 of the *vgsc* gene and an upstream intron. F1534C mutation was detected in five countries: Greece (Kavala: 10%, Chania: 13% and Athens: 47%), Georgia

(Batumi: 4%), Albania (Saranda: 3%), Romania (Bucharest: 2%), and Spain (Sevilla: 2%). The widespread geographic distribution of the F1534C mutation coupled with the haplotype analysis suggest multiple mutation events in the origin of these mutations with differentiated haplotypes including heterozygotes and mutant, raising the alarm for the establishment of IR in European *Ae. albopictus*.

P26: Development of Genetic Sexing Strains for genetic control of *Aedes albopictus*

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Ae. albopictus is an invasive species as well as a major vector of arboviruses worldwide. Given the environmental threat posed by insecticides and the emergence of resistance rendering them inefficient, alternative vector control tools are needed. Genetic control relies on the release of large amounts of lab-produced males competing with field males for mating with females. Such approaches have proven very efficient in eliminating *Ae. albopictus* populations in several locations. However, they remain expensive to settle, in part due to the poor efficiency of the sex separation step during mass rearing. In order to tackle this limitation, we developed two genetic sexing strains based on fluorescence markers linked to the m and M sex loci, allowing isolation of transgenic males. Furthermore, we show how combining these sexing strains allows the production of non-transgenic males. Scaling-up would allow 100,000 neonate male larvae to be sorted in under 1.5 hour with 0.01-0.1% female contamination, with resulting males presenting similar survival and flight ability as laboratory-reared wild-type males. By facilitating the sorting of transgenic or non-transgenic males, such Genetic Sexing Strains should enable a major upscaling of genetic control programs against this medically important vector.

P27: Vector risk prevention by mosquito monitoring using a connected Qista traps

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The mosquito family (Diptera: Culicidae) encompasses more than 3592 valid recognized species. Some of them remains the deadliest fierce hunters of human beings on the planet. Malaria, Zika, Dengue, Chikungunya, Yellow fever, West Nile, and the list is not over, are the main diseases implicating both the twin feared actors; mosquito and pathogens. Because of that, several methods are employed to fight against mosquito and their vector role. The Qista mosquito solution is based on the dispersion of recycled carbon dioxide to simulate human breath and release of an olfactory lure to simulate human body scent. The operating program can be controlled remotely thanks to a Qista application connected to a main board installed in the trap. Also the meteorological data namely temperature, barometric pressure and humidity can be recorded each hour the day. Using sensors, the Qista application allows users to monitor the captures in the real time. This “big health data” collected on more than 6000 traps set around the world give us a valuable information on the evolution of mosquito populations, thus allow us to model the proportion of mosquito populations in an area and establish an effective control program against mosquito and their vector role.

P28: “The Sterile Insect Release Technique (SIT) in Greece: results and challenges”

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In the context of this integrated management of *Aedes albopictus*, the synergistic action of the sterile Insect Technique (SIT) with the simultaneous implementation of educational visits ("door-to-door") for the information of the citizens was evaluated. “Door to Door” (D-to-D) was aimed at reducing breeding sites in private premises & Sterile Insect Technique (SIT) at reducing the reproductive potential of adult females. The application has already begun since the autumn 2018 in the area of Vravrona (Municipality of Markopoulo) against *Aedes albopictus* (Diptera: Culicidae). The implementation of D-to-D method a number of questionnaires were

distributed, based on KAP methodology (Knowledge-Attitudes-Practices). In SIT trials, 3000 sterile males/ha were released once a week to overwhelm the wild population. The results from D-to-D revealed visits led to a significant reduction in the density of eggs. The results from hatching protocol revealed a significant reduction of hatchability in the area where the sterile technique was applied in comparison to control area. The initial combination of SIT and door-to-door methods resulted in noteworthy results in the management of *Ae. albopictus* in urban areas. Based on good results, further research is needed to expand door-to-door strategy as well as to optimize quality SIT parameters.

P29: Tensorflow and vector fertility: the automatic classification of pyriproxyfen-damaged mosquito ovaries

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Pyriproxyfen (ppf) is being trialled as a tool to overcome pyrethroid resistance, inducing sterility which is measured by scoring oviposition or inspecting ovaries, depending on the setting. Ovary scoring even by trained experts is inefficient, inconsistent, difficult to replicate and its accuracy is hard to substantiate. Therefore, a freely available alternative method for the accurate, quick and automatic classification of ovary damage is required. Using the tensorflow library within python, a resnet-50 convolutional neural network (cnn) was pre-trained using the imagenet dataset. This cnn architecture was then repurposed and measured using a novel dataset of 163 dissected ovary images with known fertility status and ppf exposure. Data augmentation maximised the training dataset and produced 2,552 random images. A test set of 47 images was used to measure accuracy. The model produced an accuracy score 94% and a rate of true positive against false positives of 90%, analysing the 47 images in the test set in 12.83 seconds. This approach can thus achieve an acceptable level of precision, in a quick, robust format and has the potential to be easily distributed in a practical and accessible manner. With further photosets this tool can be expanded to additional mosquito species and applications.

P30: Protection against *Aedes albopictus*: A network of capture traps for mated adult females in search of blood

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The presence of *Ae. albopictus* is synonymous with nuisance when female mosquitoes are looking for a prey to secure a blood meal. This search mainly involves the sensory and nervous systems of the mosquito, which participate in the localization and identification from the CO₂ and VOCs released by the prey. The traps on the market are relying on a luring strategy and offer a method capable of avoiding ecological damage. However, these individual traps prioritize capture, but do not provide complete protection, because of limitations linked to the positioning of the individual traps caused, e.g., by the direction of the wind or by human attractive cues. Evidently, these traps were originally developed as sampling and monitoring tools. In order to address these shortcomings and fight effectively against the nuisance of *Ae. albopictus*, Dipteratech has developed a belt system to control a network of traps placed around infested areas. This network makes it possible to capture culicidae and eliminate the local colonies, preventing reinfestation. It therefore provides a real protection forming a barrier around the area. This concept can naturally operate in synergy with an integrated pest management program, which represents an advantage for the control of *Ae. albopictus*.

P31: Identification of an immuno-epidemiological biomarker of cat exposure to *Aedes* mosquito bites

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Aedes albopictus, vector of the chikungunya, Zika and dengue viruses, has colonized 67 departments in mainland France. Established in Occitania since 2011, it has since been responsible for 12 native cases of chikungunya and 15 native cases of dengue (917 native cases in Europe, including 79 cases in

France). The tiger mosquito is also a vector of *Dirofilaria* species, cause of cardiopulmonary heartworm disease (inducing fatal cardiorespiratory failure without treatment) and subcutaneous heartworm disease in dogs and cats. The risk of exposure to *Aedes* bites - allowing transmission of infectious agents - is currently estimated by mosquito abundances. Individual and population assessment of human-vector contact is therefore generally extrapolated rather than measured. An indicator for monitoring exposure by biomarkers of exposure to vector bites has therefore been developed and validated in human individuals. This biomarker is based on the quantification of antibody (Ab) responses to different antigens in *Aedes* saliva injected at the time of the bite, by means of an immunological ELISA test. In particular, the specific Ab response against a salivary peptide (Nterm-34kDa-*Ae. aegypti*) has been validated as a reliable quantitative biomarker of human exposure to *Aedes*. *Aedes albopictus* is known to be particularly mammophilic and anthropophilic. However, recent studies show that the proportion of blood meals taken from humans in peri-urban areas can be significantly lower than those taken from domestic cats. Domestic cats are the most common pet in France and can't be protected from mosquito bites (no effective repellent is approved for cats). Thus, the cat can be considered as a relevant sentinel animal for human exposure to mosquito bites, in a "One Health" approach. Our main objective is to determine whether cats experimentally exposed to *Aedes* bites can develop a specific Ab response to a salivary antigen (Nterm-34kDa-*Ae. Aegypti*). This first step will allow us to validate this salivary peptide as a quantitative and temporal biomarker of exposure to *Aedes albopictus* bites in cats.

P32: Serosurvey following the first vector-borne transmission of Zika virus in Europe

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In 2019, the first vector-borne transmission of Zika virus in Europe was documented in southern France. Active case finding allowed to identify a cluster of 3 autochthonous cases located in a residential area, colonized by *Aedes albopictus* since several years. The primary case was not identified, but

analysis conducted by the national reference laboratory for arboviruses suggested a Southeast Asia origin. To determine the true extent of the transmission and the proportion of asymptomatic and paucisymptomatic infections, a serosurvey was conducted. Capillary blood from consenting household members, and information on their medical and travel histories were collected. Recent infections were identified using IgM and IgG Flavivirus ELISA, followed, when positive, by plaque reduction neutralisation tests on serum against Zika, dengue and West Nile virus. The survey was conducted among 61% of households in the study area (89/146) and 86% of eligible persons were sampled (165/192). Eight persons had positive IgG anti-flavivirus serology. All had a history of travel to intertropical areas, of dengue fever, or yellow fever vaccination. The seroneutralizations performed confirmed previous dengue infections. No additional cases of Zika virus infection were identified through the seroprevalence survey, suggesting a limited focus of transmission.

P33: IgE response against *Aedes albopictus* salivary protein in allergic patients

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Mosquito bites following first exposure cause an immune response driven by sensitization to mosquito salivary proteins mediated by immunoglobulins, i.e., Immunoglobulin (Ig) G and IgE, and immune cells. In particular, IgE and IgG are involved in early responses, and IgE saliva-specific antibodies are thought to be the main cause of allergic reactions. Clinical diagnosis of mosquito allergy can be assessed using various diagnostic tools, but the use of whole-body extract from mosquitoes as source of allergens displays several limits in terms of sensitivity and specificity. On the contrary, saliva or salivary gland extract (SGE) preparation result in a more accurate diagnosis of mosquito allergy, but their preparation is laborious, time consuming and requires large amounts of mosquitoes. Therefore, recombinant mosquito salivary allergens could be a powerful alternative to these preparations. In the present study we investigate the use of three recombinant proteins secreted in *Ae. albopictus* saliva (LIPS-2, Ag5-3 and D7) to detect IgE response in subjects affected by mosquito allergy according to clinical diagnosis compared to controls from the same population.

P34: The Citizen Science Project 'Signalement-moustique' Helps Monitor the Distribution and Spread of *Aedes albopictus* in France

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Since his arrival in France in 2004, the tiger mosquito, *Ae. albopictus*, is still spreading in France, to be established in 67 French metropolitan departments out of 96 in 2022. In 2014, whereas *Ae. albopictus* was implanted in 18 departments, the French Ministry of Health has called for the creation of a website www.signalement-moustique.fr in order to allow citizens to signal the presence of the tiger mosquito, by submitting mosquito pictures or specimens collected in their private surroundings, and so contribute to its monitoring. This helps Health Authorities to follow the front line of colonization, the expansion of the colonized area and the seasonal dynamics of the tiger mosquito. Indeed, managers from public authorities and vector control operators use the data as source of information for implementing monitoring and vector control measures. So far, since its establishment more than 60 000 reports have been notified on the website. We analyzed these reports over the 2014-2019 period, so around 43 000 reports. Among these reports, 4 548 were outside of the already known colonized area and helped Health Authorities. The data suggest that a citizen science project such as the 'Signalement-moustique' may aid in detecting changes in the *Ae. albopictus* spreading and can therefore be used to guide the design of more targeted field surveillance activities. Following analysis of the above data, we showed that citizen-based surveillance provides a flexible, scalable, and cost-effective approach that can increase the rapidity and accuracy of invasive species detection.

**P35: The social construction of robust citizen knowledge
regarding the prevention of infectious risks linked to *Aedes
albopictus* (Tiger Mosquitoes) in Occitania**

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In France, since 1998, the tiger mosquito has been subject to increased surveillance. Identified as a health risk by the institutions in charge of regulating this problem, it appears more and more as a simple nuisance for most citizens. Taking the Occitanie region as a case study, this thesis will aim to question the methods of construction of this "public problem", between nuisance and risk around the tiger mosquito. By focusing on the social and cognitive citizen processes (beliefs, convictions, practical commitments, etc. integrated by different citizen profiles), this research will highlight the differentiated relationships to the nuisances and health risks of *Aedes albopictus* according, in particular, to categories of citizen knowledge. At the crossroads of the sociology of health, the environment and public action, this study will begin with the design of an Occitan panorama of the actors mobilized on the issue of vector risks. Then, a survey will be carried out by semi-structured interviews with the various actors involved in the issue of arboviruses, and more particularly the tiger mosquito. Finally, a quantitative questionnaire will make it possible to refine the research by more systematically linking the profiles and knowledge of citizens. On the other hand, several observations will be carried out as part of a participatory science system concerning vector control in the Occitanie region supported by the RIVOC Key Challenge.

P36: Social mobilization in tiger mosquito control

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Social mobilization is an essential tool to control tiger mosquito. Since many years, EIRAD (Entente Interdépartementale Rhône-Alpes pour la démoustication), a French public mosquito control organisation, assists public authorities with training of and technical assistance to local government, municipal building entomological diagnostics, and through house-to-house actions with residents. To go further in this approach, two experiments were carried out as part of the house-to-house actions. The first concerns the creation of an incentive kit for good practices in collaboration with behavioural psychologists. New tools like engagement charter, calendar, sticker... are distributed after the diagnostic of the house. The second concerns the providing of gravid mosquito traps to the inhabitants of a commune, once all the larval breeding sites had been eliminated. The first results of these experiments are encouraging and appreciated by the inhabitants.

P37: *Aedes albopictus* control: What is the best scale to strengthen field actions and political decisions?

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The Asian Tiger Mosquito (*Aedes albopictus* - *Stegomyia albopicta*) is spreading around the world and has now established itself in many European countries. This invasive species was intercepted for the first time in 1999 in the West of France on a site of imported tires. Since its establishment in the south of France in 2004, *Aedes albopictus* has now definitively colonized 67 French departments (3965 municipalities) in 2021. Since 2010, autochthonous cases of dengue or chikungunya have been observed every year in France. The risks of transmission of vector-borne diseases have led to the necessary resilience and the implementation of new vector control strategies. However, even if the bites caused by *Aedes albopictus* represent a real annoyance that need political answers, this nuisance can be an opportunity to mobilize community participation to enhance passive mosquito surveillance and control in the field using natural predators, physical barriers, biocides or traps.

More or less 80% of tiger mosquito breeding sites can be eliminated by covering, tidying up and emptying all objects that may contain rainwater. The main means of bringing about a change in behavior is through information and education of people based on all means of communication. The combined involvement of all stakeholders at each level, from the Ministry of Health, through municipalities and mosquito control services, to owners, is the central axis of integrated mosquito control.

P38: Dengue circulation in La Réunion island since 2018

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We presented the epidemiological and entomological situation of dengue in La Reunion Island. Since 2018, five epidemics occurred, but quasi no circulation has been observed between the seasonal epidemics. Several serotypes have circulated: dengue 1, 3 and 4. In 2021, an intensive and dramatical epidemic occurred, with the first severe dengue cases: dengue shock syndrome and also neurological and ophtalmological complications. Vector control strategy in La Reunion Island is well organized with a reactive strategy based on the automatical spatio-temporal reporting of the dengue cases from the labs to the vector control strategy unit. This allows a reactive strategy in the field.

P39: Urban greening and potential impacts on vector-borne diseases in Occitanie

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- 7 ESPACE DEV (IRD), Montpellier

Worldwide, the percentage of people living in urban areas is expected to reach 70% by 2050. This will result in expansion and/or densification of urbanized areas. This process leads to environmental and behavioral changes that affect the well-being of urban population and sustainability of the cities. Introducing nature into cities may improve the situation by reducing heat islands, restoring biodiversity, providing different ecosystem services. But cities are a very complex, fragmented, and open environment, with high concentrations of populations (human, animal, and plant) and many exchanges. Their functioning is still poorly understood, particularly with regard to vector-borne diseases. Our study aims to assess the possible impacts of greening on vector-borne diseases, and vectors, including *Aedes albopictus*, for humans, animals and plants in Montpellier and Toulouse. Through a One Health-type approach, the objective is to develop an integrated vector risk monitoring system will make it possible to propose control and prevention actions shared by all the actors concerned.

P40: The Global Vector Hub - building entomological capacity worldwide and improving epidemic preparedness

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The aims of the Global Vector Hub (GVH) are to assist in capacity building for vector control globally, establish a community of practice for vector control interventions, and enable stakeholders to make evidence-based decisions. The main audiences of the GVH are public health officials, vector control agents and vector researchers. The GVH consists of a community-led, online, open-access resource to provide comprehensive information on vector control and vector biology. This includes geo-tagged entomological data

(including abundance data, surveillance for insecticide resistance, and pathogens vectored) and epidemiological data, a searchable registry and worldwide network of vector researchers and vector controllers, and a comprehensive resource database of training and educational materials, vector control guidelines and research tools.

**P41: A taxon-enriched phylogenomic approach to disentangle
Aedes albopictus evolution**

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Phylogenomics has the potential of identifying the genetic basis of *Aedes albopictus* invasive traits by identify for example gene family expansions, events of positive selection, and generation times. *A. albopictus* genome has been however compared solely with relatively distant *A. aegypti* and with distant non-*Aedes* mosquitoes: these comparisons cannot discriminate specific adaptively acquired characters from those shared with closer related (sister-species). To increase the taxon sampling in the *Aedes* clade, we present genome data for *Aedes japonicus* and *Aedes koreicus*. The data skimming of these genomes allow us to perform mitogenomic, phylogenomic, and clock studies on a taxon enriched sample and to define with more confidence genetic traits which are unique of subgenus *Stegomyia* and (sub?)genus *Ochlerotatus*. To improve the resolution power of phylogenomic studies, we further advocate the sequencing of the genome of *Aedes flavopictus* (the sister species of *A. albopictus*) and in general the need for enlarging the genome sampling within Aedini: such enhanced comparative framework will provide more precise information for understanding the intimate biology of *A. albopictus* and ameliorate its management.

P42: The Digital Entomology Laboratory: the Expert Network of Mosquito Alert

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Mosquito Alert is a citizen science platform aiming to generate an alert system helping in the surveillance and management of disease-carrying and invasive mosquito species. Citizen scientists provide through a specific cellphone app geo-localized reports containing images of targeted mosquito species, breeding sites and biting behaviour. The reports of adult mosquitoes go to the Digital Entomological Laboratory (DEntoLab) for a validation process, which is currently performed by a group of more than 64 expert entomologists from different countries of Europe. Some of these countries have a national supervisor and there is also a general coordinator. The validation process is performed independently by 3 experts and an average classification is automatically calculated for each report. Artificial Intelligence (AI) algorithms are being integrated within the system to make classification faster and to relieve experts workload. The D-Entolab sets our “human-in-the-loop” strategies to train AI in order to get more information in the images. For example, information on targeted mosquito body parts, on blood-feeding stages, or on image background environments (in-out home). This information would allow not only to improve the taxonomic level of our AI, but also provide new AI on human-mosquito interactions and host-vector network dynamics. Any citizen science project needs to promote participation in its user community using outreach, rewarding and gamification strategies. As entomologists participate on a volunteer basis it is important to work also in expert community building, to motivate expert participation, make the

system efficient in near real-time, and long-term sustainable. For this, in addition to enhanced internal communication, working groups and workshops are set up. Also, a crediting system for a fair acknowledgement of the experts activity is being studied.

Thank you for your
participation

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