ABSTRACTS:

Chemical Ecology of Disease Vectors:

Oral Presentations

In programme order

Session 1

Insights into Use of Semiochemicals for Management of Disease Vectors in Kenya

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In recent years, previously controlled vector-borne diseases have resurged or remerged in new geographic areas. Few vaccines are available to control these vector-borne diseases. Reports of resistance to drugs and insecticides by vector-borne pathogens and vectors, respectively, have become of major concern. To effectively tackle these vector-borne diseases, we at the International Centre of Insect Physiology and Ecology, Nairobi, Kenya, argue in a recent article entitled "Grand Challenges in Vector-Borne Diseases Targeting Vectors" published in *Frontiers in Tropical Diseases*, that given the existing constraints, development of vector control tools should be intensified to reduce vector-human contact. We identified several priority research areas to facilitate the development of innovative control tools, among them the chemical ecology of disease vectors. In this lecture, I will highlight some of the progress made in the development of these tools based on understanding and exploitation of weak links in the chemical ecology of vector-host interactions, focusing on a few diseases; sleeping sickness, malaria, dengue and yellow fever, Rift Valley fever and leishmaniasis in Kenya. I will also highlight a few research areas to aid development of more effective semiochemical-based tools for disease vector management.

Keywords: disease vectors, sleeping sickness, malaria, leishmaniasis, Rift Valley fever

Non-human primate and human-derived attractants for *Aedes* mosquitoes

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Yellow fever (YF) and dengue (Den) share a common niche mainly transmitted by *Aedes* (*Stegomyia*) species. Surveillance of vector populations is a critical component of risk assessment for YF and Den transmission and outbreaks. Implementing this effectively requires the use of efficient trapping tools for monitoring vector populations which can be achieved by employing lures through knowledge of their blood-seeking behavior. Blood-seeking mosquitoes use host-derived cues including breathe (e.g. CO_2) and body chemicals for host location. So far, identification of host-derived odors as attractants has been attempted for domestic anthropophilic *Aedes aegypti*. However, similar data remain lacking for non-human-biting *Aedes* vectors in a sylvatic setting where non-human primates (NHP) serve as reservoir hosts. This presentation will highlight progress in our efforts to develop attractive lures for improved surveillance of adult *Aedes* vectors of YF and Den in both sylvatic and domestic environments, employing chemical ecology approaches and a series field experiments.

Keywords: *Aedes*-borne viruses; host-seeking; lures; sylvatic environment; domestic environment

Malaria mosquitoes take the piss

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Feeding on the secretions or the excrement from vertebrates, behaviours included in the broader term 'puddling', has been described in a range of herbivorous and detrivorous terrestrial arthropods, but to date not in hematophagous insects. Supplementary feeding by females of the malaria mosquito *Anopheles arabiensis* on cattle urine, a rich source of nitrogen compounds, differentially enhances survival, flight behaviour and reproduction, an effect dependent on the age of the urine. These results extend our current understanding of which nutrients are limiting to these disease vectors. Female *An. arabiensis* are attracted to cattle urine odour, a behavioural response modulated by the age of the urine, reflecting the observed effects on life history parameters. Chemical, electrophysiological and behavioural analyses allowed us to identify bioactive volatile compounds, which when presented as blends in their natural ratio reproduce the behavioural response to differently aged cattle urine. One of these blends attracts both host-seeking and blood fed mosquitoes in the field. When assessed in a large-scale longitudinal study, a village equipped with outdoor traps baited with this blend, captured substantial numbers of mosquitoes leading to >60% and >80% reduction of indoor host seeking and resting *An. arabiensis*, respectively, and ca. 60% reduction of malaria prevalence compared to that of the control village. As a whole, this study is a proof of principle that an increased understanding of the ecology of disease vectors can lead to effective control measures of vectors and the disease they transmit.

Keywords: puddling, *Anopheles*, chemical ecology, attraction, vector control

Age matters: gene regulation in the mosquito antenna during the first gonotrophic cycle

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Mosquito behaviours associated with maturation, feeding and reproduction directly affect vectorial capacity. Odours mediate each phase of the gonotrophic cycle from host blood seeking through post-blood meal refuge seeking, oviposition site seeking and oviposition, and finally to post-oviposition reassertion of host seeking, while sugar seeking continues throughout. The age- and blood meal-induced regulation of chemosensory, neuromodulatory and other signal transduction genes was investigated in the antenna of female mosquitoes during maturation (1 and 3 days post-eclosion), and subsequently every 24 h from 5 to 10 days post-eclosion from two cohorts: 1) non-blood fed and 2) blood fed. Using a transcriptomic approach, we observed a concerted regulation of multiple genes within the sensory pathways of the antenna, which likely play a key role in modulating the behavioural changes observed with age and post-blood meal. Functional characterization of the proteins generated by the genes-of-interest identified in this study may provide a better understanding of the regulation of gonotrophic feeding and a pool of potential targets for vector control strategies.

Keywords: gene expression; olfaction; transcriptomics; blood feeding; host seeking

Life scientists in chemical ecology can benefit from psychology's responses to its own 'reproducibility crisis'

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Reproducibility failures have affected all parts of the life sciences relevant to chemical ecology, from animal behaviour to molecular biology. Psychologists have responded constructively and creatively to their own field's very public 'reproducibility crisis'. The solutions include new ways of doing experiments, such as Registered Reports and aspects of Open Science. To illustrate some of our own challenges in chemical ecology, I will use the story of the 'putative human pheromones' androstadienone and estratetraenol which, despite never having been shown to be pheromones, have been the subject of some 60 studies claiming 'significant' positive results. These are quite possibly false positives, part of the 'reproducibility crisis', sadly common in the rest of the life and biomedical sciences, which has many instances of whole fields based on false positives. Chemical ecological research would benefit from vigorously adopting the proposals made by psychologists to enable better, more reliable science, with an emphasis on enhancing reproducibility. A key change is the adoption of study pre-registration and/or Registered Reports, which will also reduce publication bias. A growing number of journals covering chemical ecology offer Registered Reports, including *BMC Biology, Nature Communications, Scientific Reports*, and *Royal Society Open Science*.

Keywords: pre-registration; reproducibility; false positives; p-hacking; open science

The ionotropic co-receptor IR8a is required for the expression of robust sexual behavior in males of the Chagas disease vector *Rhodnius prolixus*.

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The ionotropic receptor co-receptor IR8a is involved in diverse chemosensory-driven behaviors in arthropods. It has been reported as necessary for host-seeking in mosquitoes, foraging in *Drosophila melanogaster,* and shown to have a significantly higher antennal expression in males of the copepod *Eurytemora affinis*. Increased antennal expression of IR8a was also recently reported for male *Rhodnius prolixus*. Our current results show that significantly decreased *RproIR8a* expression (RNAi) affects the sexual performance in males of this species. We significantly knocked-down the expression of *RproIR8a* in male adults and subsequently used them to test their sexual responses. Firstly, *RproIR8a* knocked-down males showed a significantly decreased tendency to leave shelters in the presence of females in comparison to intact males. Furthermore, a lower proportion of *RproIR8a* knocked-down males approached females in the same experiment. A second experiment showed that *RproIR8a* knock-down induced a decreased mating frequency in single pair assays. This is the first study to incriminate *IR8a* in insect sexual behavior. Future studies should determine which sensory processes affected by *RproIR8a* knock-down relate to the negative effects here described in males of this species.

Keywords: triatomines, sex, pheromones, receptors, olfaction

Tick Repellents: Modulators of Electrophysiological and Behavioural Activities

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Understanding tick olfaction has relevant implications in animal and human health for the development of repellent alternatives. The effects on tick chemosensory system of long-term exposure to repellents is unknown, and no studies have investigated potential long-term disruption and inhibition of tick abilities to detect hosts. We investigated whether pre-exposure to repellents impact the chemosensory system of ticks. We recorded the electrophysiological response of *Ixodes scapularis* female ticks to a known attractant and host volatile (i.e., butyric acid), before (i.e., pre-) and after (i.e., post-) exposure through fumigation to selected known repellents (i.e., lemongrass, DEET). Behavioral Y-tube bioassays were performed to evaluate potential changes in attraction post-exposure to butyric acid. After 20 minutes of fumigation, exposure to repellents significantly inhibited tick's ability to detect host volatiles by decreasing electrophysiological and behavioural activities. These results demonstrate repellents can act as inhibitors, modulating tick chemosensory activity, and provide important information in better understanding mode of action of tick repellents.

Keywords: *Ixodes scapularis*; tick chemosensory system; essential oil; exposure; inhibition

Functional analysis of two odorant receptors related to host seeking in *Aedes aegypti*

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The mosquito *Aedes aegypti* is the primary urban vector of several highly infectious human pathogens. Disease transmission heavily relies on the ability of the female to locate and feed on the blood of infected hosts, which are primarily odour-mediated behaviours. Olfactory sensory neurons, housed in sensilla, express a wide array of chemosensory genes that are likely to play central functions in these behaviours. The emergence of heterologous systems to express and screen odorant receptors (ORs) with known behaviour-modulating volatiles has permitted the reliable identification of key ligands for these receptors. Three ORs-of-interest, *AaegOr4, AaegOr103* and *AaegOr117*, were previously described by our group as potentially involved in the evolution of human host preference and the modulation of onset host seeking in female *Ae. aegypti*. While *AaegOr4* was upregulated in human-preferring mosquitoes and showed a consistent response to sulcatone, a volatile compound highly abundant in human odour, the function of the two other receptors remains unknown. Through GC-SSR, we monitored odour-evoked responses in *AaegOr103* and *AaegOr117,* heterologously expressed in the *Drosophila* ab3A empty neuron system, and revealed both antennal inhibition and activation to certain volatile compounds collected from humans and host plants. We speculate that these two receptors are candidate genes involved in the evolution of host preference and the modulation of odour-mediated behaviours in *Ae. aegypti.* A better understanding of the function of these and other ORs could enable the development of future control strategies against vector borne diseases.

Keywords: deorphanization; enantiomers; olfaction; reverse chemical ecology; sensillum

Electrophysiological responses of *Ixodes scapularis* to host volatiles

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Ixodes scapularis is an important vector of *Borrelia burgdorferi*, the causative agent of Lyme disease, in North America. In recent years, the threat of disease transmission has risen significantly, resulting in an increased demand for environmentally safe, tick repellent products. To better understand and potentially disrupt host seeking behavior, host odorants can be identified and characterized, allowing for a more in depth understanding of what volatile organic compounds promote tick host-location. We identified key odorants/blends by collection and chemical characterization of volatile compound (VOC) from hosts (e.g., humans, cats, dogs, etc.) and the mechanism by which ticks detect them. Through a novel electrophysiological approach, we recorded tick responses to host VOCs using gas chromatography linked electrotarsography (GC-ETG). After identification of biologically-active chemicals , we further tested relative sensitivity to homologous series of carboxylic acids, aldehydes, and hydrocarbons at different concentrations. Ticks responded most strongly to compounds with 4-6 carbons, denoting a specific pattern of sensitivity. Behavioural assays assessed tick response to stimulation with electrophysiologically-active components. These results will contribute to defining key host volatiles that trigger host-seeking behaviour.

Keywords: electrophysiology; tick; host volatiles; carboxylic acids; aldehydes

ABSTRACTS:

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Poster Presentations

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Poster Session 1

Head Louse Feces: Chemical and Behavioural Analysis

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Human head lice *Pediculus humanus capitis* (De Geer) (Phthiraptera: Pediculidae) are insect parasites closely associated with humans, feeding on the blood of their hosts and causing them skin irritation and probable secondary infections. Despite being a severe nuisance, very few studies have reported on intraspecifc chemical communication in head lice. Here, we evaluated the behavioral responses of head lice to the volatile compounds and solvent extracts from their feces. We also chemically analysed the main volatile components of these feces by CG-MS and those of the feces extracts by HPLC-MS. Head lice were attracted to the methanol extract of their feces but not to the hexane or dichloromethane extracts, suggesting the polar nature of bioactive chemicals present in head louse feces. Follow-up chemical identifications, in fact, showed the presence of hypoxanthine, uric acid, and another purine tentatively identified as either guanine or iso-guanine. Additionally, head lice were significantly attracted by volatiles emitted from samples containing feces. The volatiles emanated from feces alone contained 19 identified substances. The major compounds found were decanal, nonanal, hexanal, and acetic acid, together representing approximately 60% of the identified compounds. This work represents the first chemical evidence of intraspecies communication among head lice. The results support the existence of active substances present in the feces of *P. humanus capitis* that may be involved in its aggregation behaviour.

Keywords: Head lice, feces, behavioral activity, chemical analysis

Landscape-Vector-Virus Interactions: Do farms surrounded by diverse landscapes benefit from enhanced virus suppression?

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It has been well documented that agricultural fields surrounded by a more diverse and/or more complex landscape often benefit from an increase in the provision of ecosystem services, including the suppression of agriculturally important herbivorous insect species. However, it is unclear whether this benefit extends to the suppression of plant diseases that are transmitted by these herbivorous insects. Barley yellow dwarf virus (BYDV) is an important aphid-vectored plant virus that can cause significant yield losses in winter cereal crops. BYDV control is generally achieved through the suppression of BYDV vectors via pesticide application. However, emerging insecticide resistance in BYDV vectors has increased the need for alternative avenues of BYDV vector control. One potential avenue for alternative BYDV vector control is to promote the delivery of ecosystem services that facilitate natural pest/virus suppression. In this project, 17 winter wheat fields, selected along a landscape diversity gradient, were assessed for BYDV symptoms at multiple timepoints from tillering and during stem elongation. The extent of BYDV infection was determined across each field and was assessed in relation to 1) landscape diversity (i.e. the diversity of land use types around fields), 2) landscape complexity (i.e. amounts of non-crop habitats around fields), and 3) natural enemy activity. The results of this project will give further insight into landscape-vector-virus interactions and will improve our understanding of the potential to promote beneficial ecosystem services through increased landscape diversity.

Keywords: Ecosystem Services; Entomology; Landscape Ecology; Pest Suppression; Virology

Repellent and acaricidal effects of basil essential oil and rock dust on ticks

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Ticks, such as blacklegged ticks (*Ixodes scapularis*) and American dog tick (*Dermacentor variabilis*) are vectors of several pathogens that negatively impact animal and human health. In recent years due to global warming, the threat of disease transmission has risen significantly, resulting in an increased demand for environmentally safe, tick repellent and acaricidal products. Natural products, such as essential oils and inert rock dust, are prospective alternatives to manage these pests. Basil (*Ocimum basilicum*, L.) has been reported to have promising pest repellent activity. We extracted and characterized essential oils from different basil varieties, and tested them for long-term repellent activity towards nymphs of blacklegged ticks using horizontal bioassays at different concentrations. In addition, we combined basil essential oils with an inert material (i.e., granite rock dust) with known insecticidal properties to assess acaricidal activities against adult ticks. Among the tested basil varieties, Jolina essential oils (15% v/v) repelled 96% of tested ticks up to 2 hours post-treatment. In acaricidal tests, the combination of essential oils from Aroma2 var. at 10% w/w with rock dust resulted in 100% tick mortality after only 24 hrs post-exposure. The use of essential oils alone, and in combination of rock dust, represents an innovative and environmentally-friendly approach to manage ticks and the spread of vector-borne diseases.

Keywords: ticks; essential oil; acaricidal; repellent; granite dust