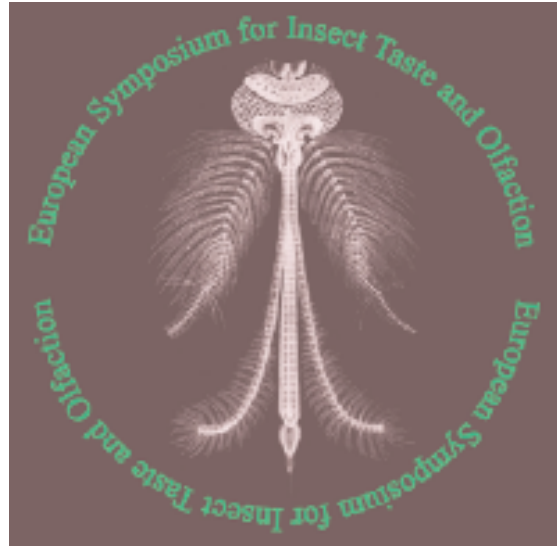


ESITO 2025

Friday 19 September 2025 - Wednesday 24 September 2025

Villasimius, Italy



Book of Abstracts

Talk abstracts

Keynote Lecture

The chemical language of insect-microbe symbioses

Author: Martin Kaltenpoth¹

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Insects represent the most diverse animal class on earth, and their evolutionary success is at least partly due to their ability to engage in manifold interactions with beneficial microorganisms. These symbiotic microbes can live intra- or extracellularly in their insect hosts and provision limiting nutrients, digestive or detoxifying enzymes, or protective chemicals that enhance host fitness. While research of the past decades has uncovered the astonishing taxonomic and functional diversity of insect-microbe associations, the chemical and molecular basis underlying their establishment, maintenance, and transmission remain incompletely understood, especially due to the scarcity of experimentally and genetically tractable intracellular symbioses. Grain pest beetles, especially the saw-toothed grain beetle *Oryzaephilus surinamensis*, present excellent study systems, as they harbor intracellular symbionts that can be experimentally eliminated without killing the host. Using a combination of chemical analytics, molecular tools and bioassays, we have uncovered the role of the symbionts in the biosynthesis of tyrosine and its impact on the beetle's cuticle biosynthesis and defense against enemies. Furthermore, we managed to introduce a genetically tractable intracellular bacterium into *O. surinamensis* that now allows us to study the molecular basis of symbiosis establishment as well as the interaction between the newly introduced bacterium and the ancient endosymbiont. The results provide interesting first insights into the molecular host-symbiont interplay as well as the evolutionary steps leading to the establishment of novel symbioses.

Chemical Ecology

Comparative chemosensory systems in *Aedes* mosquito larvae: The role of olfaction in foraging behavior

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The invasive Asian tiger mosquito, *Aedes albopictus* (Skuse), and yellow fever mosquito, *Aedes aegypti* (L.) are known to compete for resources during the larval stage, often resulting in the ecological displacement of *Ae. aegypti* by *Ae. albopictus*. The chemosensory system plays a pivotal role in larval foraging behavior and may contribute to the competitive advantage. Here, we employed comparative transcriptomics and functional characterization of odorant receptors (ORs) to investigate species-specific differences in larval olfaction. Notably, we uncovered functional variation within the conserved olfactory indole receptor clade, indicating distinct ecological adaptations across species and life stages. We also developed a novel approach to functionally characterize the larval sensory cone and mapped its receptor neuron projections to two key brain regions: the antennal lobe and the subesophageal ganglion. This study provides new insights into the molecular and neural basis of chemosensory-driven behavior in mosquito larvae and highlights the potential role of olfaction in shaping interspecies competition and ecological success.

Chemical Ecology

Hawkmoths can smell with grooming organs on their legs

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The antennae are the primary olfactory organs of insects, though other appendages, such as mouthparts and the female ovipositor, can also detect odors. A prerequisite for the olfactory function of an appendage is the presence of sensilla with porous walls and the expression of chemosensory receptors by sensory neurons housed in these sensilla. In the tobacco hawkmoth, *Manduca sexta*, we demonstrate that the epiphysis, a small process on the tibia of the forelegs that is used to clean the antennae, is an olfactory organ. The epiphysis carries approximately 150 putative olfactory sensilla and expresses numerous chemosensory receptor genes. We identified the expression of *ORCo*, the obligate co-receptor of odorant receptors (*ORs*), as well as 54 tuning *ORs*. Additionally, the epiphysis expresses 22 ionotropic receptors (*IRs*), including the co-receptors *IR8a*, *IR25a*, and *IR76b*, and 33 gustatory receptors (*GRs*). Several of these *IRs* and *GRs* had not previously been found in the antennae or other appendages. Electrophysiological recordings from isolated epiphyses revealed responses to odorants from several chemical classes, host plant leaves, and the female pheromone gland. The strongest responses were elicited by acids and the amine pyrrolidine. Epiphysectomy did not affect courtship or foraging behavior; however, epiphysectomized females were less likely to reach a host plant than controls. Our study reveals the epiphysis of *M. sexta* to be a previously unknown olfactory appendage with a broad and partly unique chemosensory repertoire. Because the epiphysis is a constitutive feature of lepidopteran insects, its olfactory function may be present in most moths and butterflies.

Chemical Ecology

Chemosensory mechanisms of temporal niche partitioning in an ecological specialist

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Animals have evolved an extraordinary diversity of behaviors to thrive in diverse environments. However, the molecular and neuronal processes shaping behavioral evolution and niche adaptation remain poorly understood. *Drosophila sechellia* is a powerful genetic model for dissecting the evolutionary and mechanistic basis of niche adaptation, as it is closely related to the cosmopolitan generalist *D. melanogaster*, but is a specialist, feeding and reproducing exclusively on *Morinda citrifolia* “noni” fruit, which is toxic for other drosophilids and more divergent insects.

In nature, noni fruit undergoes substantial changes during ripening and rotting. We have examined the olfactory responses of *D. sechellia* towards different noni stages and found that it exhibits a narrow time window of preference for ripe noni. Importantly, this stage is the most toxic for *D. sechellia*'s competitor, *D. simulans* and its predator *Leptopilina boulardi*. Through an analysis of volatiles of different noni ripening stages together with comparative single-cell transcriptomic datasets in drosophilids, we identified candidate olfactory pathways underlying this preference. Notably, we found that *D. sechellia*, but not *D. melanogaster* or *D. simulans*, expresses the receptor *Or45a* in antennae, where it mediates detection of 2-nonanone and 2-heptanone, two ketones abundant in ripe noni. These volatiles are also detected by neurons expressing *Or85c/b*, which are increased in number in *D. sechellia*. Behavioral assays using *D. sechellia Or45a* and *Or85c/b* mutants revealed that these pathways function partially redundantly in mediating preference for ripe noni. Together, our findings show that the olfactory system of *D. sechellia* has evolved to exploit a very narrow time window of its toxic host, representing an effective mechanism to enhance fitness through avoidance of competition and predation.

Chemical Ecology

Preference performance mismatch of the Eurasian spruce bark beetle, *Ips typographus*

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Ips typographus is one of the most destructive Eurasian forests pests causing major economic and ecologically losses annually. *I. typographus* typically attacks stressed trees but during mass outbreaks they are able to attack and kill vigorous trees as well. Main host of *I. typographus* is Norway spruce (*Picea abies*) but they occasionally attack trees of other species as well, possibly damaging even more forests. In our study we investigated the possibility of a host range expansion of *I. typographus* to an occasional host, Scots pine (*Pinus sylvestris*). Scots pine is widely distributed over Eurasia and mostly adjacent to Norway spruce, making it geographically a reachable host for the beetles. We performed behavioral laboratory preference assay and tracked reproductive outcome and offspring fitness in the occasional and main host. Additionally, we inoculated spruce and pine logs with symbiotic fungi of *I. typographus* and observed their growth in both trees. We conclude that *Ips typographus* can reproduce in pine trees under given circumstances with reduced performance and fitness. Males and females show attraction to pine in short range behavioral assays and no clear preference towards the main host in two-choice Assays.

Chemical Ecology

Deciphering chemical communication in an aquatic insect

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Chemical senses are crucial for species adaptation, particularly in insects, as they facilitate interactions between animals and their environment. While most insects are terrestrial, some have transitioned back to aquatic environments. This shift has likely demanded substantial adaptations to their chemosensory systems in order to cope with distinct physico-chemical constraints. How the fundamentally aerial chemosensory equipment of insects was remodelled in lineages of aquatic insects is a major and unexplored topic of interest in evolutionary ecology. We examine this question in the coleopteran suborder Adephega, which includes beetle species that exhibit a wide range of affinities to freshwater, from fully terrestrial to highly specialised aquatic species.

Our research focuses on the diving beetle *Rhantus suturalis*, a common species in Western European ponds and a significant predator of mosquito larvae. The interactions of diving beetles with other freshwater organisms have long attracted the attention of ecologists, but the underlying mechanisms, such as the nature of the olfactory signals involved, remain elusive. Using physicochemical analyses (GC-MS), we identified chemicals from ecologically relevant sources, and we confirmed their detection by *R. suturalis* antennae through electro-antennogram recordings. This provides the first insights into the olfactory capacities of these insects.

Additionally, transcriptomics revealed numerous odorant receptor genes expressed in the sensory organs of *R. suturalis*. Ongoing work aims to characterise the function of these receptors using heterologous expression in *Xenopus* oocytes and two-electrode voltage clamp. This will offer insights into the evolution of receptors that are tuned to specific compounds in aquatic environments.

Chemical Ecology

Deep behavioral phenotyping of pathogen-infected mosquitoes reveals mosquito- and pathogen-specific changes in blood-feeding behavior

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Female mosquitoes obtain a blood meal necessary for reproduction through a fascinating behavioral trajectory that culminates in the mosquito bite. The transmission of a variety of pathogens is an unfortunate by-product of this behavior. Blood feeding is contingent on the mosquito's integration of environmental stimuli and its physiological state. Pathogens infecting a mosquito impact physiology and may alter its sensory cue integration, presenting the opportunity for the pathogen to influence its transmission by affecting the blood-feeding behavior of its vector. Using custom-built high-throughput behavioral assays, we explore this hypothesis in dengue virus infected *Aedes aegypti* and several *Anopheles* species infected with *Plasmodium falciparum* and *P. vivax*. We combine the biteScope—a transparent skin mimic allowing direct quantification of mosquito biting behavior—and deep learning-based image analysis to obtain detailed behavior statistics of >2500 blood-feeding mosquitoes providing a rich perspective on activity patterns, probing behavior, and blood-feeding dynamics. Comparative statistical analysis reveals that the behavioral changes pathogens may induce differ markedly between mosquito-pathogen systems. Dengue virus, for instance, enhances *Ae. aegypti*'s propensity to engage in blood feeding nearly doubling the likelihood of infected mosquitoes to obtain a blood meal, whereas e.g. *P. vivax* has virtually no impact on the behavior of *An. stephensi*. Using an epidemiological toy-model, we explore the impact of the observed behavioral alterations on pathogen transmission.

Chemical Ecology

Oxidant pollutants and their effect on sexual communication in insects

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Insects rely on detecting airborne chemical signals, that are specific in the molecular composition, in order to induce relevant behavior. Previously, we were able to demonstrate that oxidant pollutants cause a disrupted sexual communication in *Drosophila* flies. To assess whether this effect is widespread, we conducted further experiments using such a pollutant in other insects. The results underline the importance of odorant chemical composition for the sensitive detection of behaviorally relevant cues.

Development

Development of ant olfactory systems - plasticity as a key to sociality

Author: Wolfgang Rössler¹

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All >15,000 species of ants are social and show many variations in individual behavior and colony organization. Is the evolution of the olfactory system in ants linked to sociality? Although ants express the highest numbers of odorant receptors and antennal lobe glomeruli across the insects, recent work claims that specific clusters of glomeruli and related odorant receptor repertoires in ants are not directly linked to the evolution of sociality (e.g. Gautam et al. 2024 Proc Roy Soc B; Marty et al. 2025 Proc Roy Soc B). Taking a different perspective, previous work has emphasized that behavioral plasticity represents a key factor promoting social organization and task allocation in ant colonies (Hölldobler and Wilson 2008). Does developmental plasticity in the olfactory system promote behavioral plasticity (polyethism)? Examples from different ant species suggest that caste-specific, sex-specific, and age-related developmental and adult plasticity in olfactory centers of the ant brain are linked to social organization. Cellular and molecular aspects of neuronal plasticity during postembryonic development of the antennal lobe and mushroom bodies further emphasize the potential role of differential olfactory development in polyethism. Comparative studies across social and solitary Hymenoptera are needed to understand the genetic basis underlying the evolution of developmental and adult neuroplasticity in olfactory systems and related mechanisms mediating behavioral plasticity underlying social organization.

Development

Development of GABAergic innervation within olfactory mushroom-body microcircuits of the honeybee *Apis mellifera*

Authors: Andrea Rafaela Nicolaidou¹; Claudia Groh¹; Wolfgang Rössler¹

¹ *University of Würzburg*

Social insects possess voluminous mushroom bodies (MBs), high-order sensory integration centers for multisensory integration, learning, and memory. In the honeybee *Apis mellifera*, the MBs receive olfactory input in the MB-calyx lip region. Within the lip, olfactory projection neurons (PNs) form discrete modular synaptic complexes (microglomeruli, MG), mainly with dendritic specializations from MB intrinsic neurons, the Kenyon cells (KCs). Besides excitatory olfactory input, MG in the MB-calyx lip also receive inhibitory signaling from γ -aminobutyric acid (GABA)-ergic feedback neurons from the MB lobes (type A3v). These were shown to play an important role in maintaining sparse olfactory coding by the KCs and olfactory learning and memory performances.

Our earlier studies using a combination of immunolabeling and serial section electron microscopy demonstrated a remarkable structural plasticity in olfactory MG densities and numbers associated with thermal experience mediated by brood care, adult age, task, as well as non-associative and associative sensory experience. During natural behavioral maturation from nursing to foraging, an overall reduction in MG numbers (pruning) goes along with an increase of the numbers of postsynaptic profiles per MG, resulting in an increase in the PN-KC synaptic divergence ratio by >30%. This raises the question: How do changes in innervation by GABAergic feedback neurons contribute to the plasticity in MG microcircuits?

Given the functional importance of inhibitory signaling for olfactory processing and complex learning tasks, we analyze the maturation of GABAergic innervation patterns in the MB calyx. Using consecutive age cohorts, we address quantitative aspects like the density of GABAergic terminal profiles in the calyx lip at different ages after adult eclosion. Our results show that the olfactory lip is densely innervated by profiles from GABAergic feedback neurons. Preliminary results suggest that GABAergic innervation densities remain stable during adult maturation, while the number of PN boutons decreases, thus indicating a shift in the GABAergic innervation ratio in the calyx lip. This suggests that resulting changes in GABAergic signaling may play an important role in the maturation of odor discrimination and learning abilities.

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Application**Exploiting herbivore-induced plant volatiles for crop protection****Author:** Ted Turlings¹¹ *Henan University, China, University of Neuchâtel, Switzerland, and Penn State University, USA*

Natural enemies of herbivorous pests often use plant-provided signals to locate plants that carry potential prey. Particularly intriguing are so-called herbivore-induced plant volatiles (HIPVs), which various plants release in large quantities only when they are attacked by insects. I will discuss ways in which HIPVs can be exploited for crop protection, with a focus on our efforts to utilize these highly specific plant-produced signals for the real-time detection of agricultural pests. With specific odor sensors we have been able to accurately distinguish healthy plants from plants that are under attack by insects or are infected by pathogens. We envision that these odor sensors can be placed on robotic rovers and allow farmers to determine the presence of specific pests on their crops before these pests do serious harm. The same rovers could also be used to apply biocontrol agents to control these pests, only when and where it is necessary. This should eventually result in a novel, cost-effective and sustainable crop protection strategy.

Application**From volatile to visual cues: host location and oviposition in tachinid parasitoids, with insights from *Exorista larvarum* (L.)****Author:** Maria Luisa Dindo¹¹ *University of Bologna*

Tachinid flies (Diptera: Tachinidae) are important biological control agents of herbivorous insect pests, yet their oviposition strategies and host selection mechanisms remain far less understood than those of hymenopteran parasitoids. Tachinids use both indirect and direct strategies, with the indirect ones (such as oviposition close to hosts or on host food plants) being notably more common than in hymenopterans. Indirect strategies often rely on chemical and physical cues to locate host habitats or microhabitats and are associated with high fecundity due to increased egg mortality compared with direct strategies. When host location and acceptance are carried out by parasitoid first instar larvae, the signals involved in locating a suitable host are largely unknown. In contrast, direct strategies, which are prevalent among tachinids, involve precise oviposition on or inside the host and rely on a complex interaction of olfactory, visual, and tactile cues for host location and acceptance. Herbivore-induced plant volatiles (HIPVs), host frass, host pheromones and visual cues such as host movement play key roles in host location for direct-type species like *Trichopoda pennipes* (F.), a parasitoid of stink bugs, or *Exorista larvarum* (L.), a larval parasitoid of Lepidoptera. *Exorista larvarum* can be also reared in vitro, even eggs laid outside the host, but inducing oviposition on artificial substrates remains challenging. A better understanding of the sensory and behavioral mechanisms underlying oviposition could enhance the possibility of eliciting egg laying by *E. larvarum* on selected hosts and even artificial substrates, thus facilitating its rearing and ultimately making its exploitation as regulator of target insect pests more feasible and efficient.

Application**Does the natal host species affect host choice of pupal filth fly parasitoids?****Authors:** Jean-Christophe Billeter¹; Julia M.E. Scholte¹; Leo W Beukeboom¹; Wessel R Warnders¹

¹ *Groningen Institute for Evolutionary Life Sciences, University of Groningen*

Filth flies are a common problem in the livestock industry, having negative effects on the health and wellbeing of livestock and their caretakers, leading to economic losses. With an increasing need for integrative pest management that relies less on pesticides, focus is shifting more to biological control such as the use of parasitoids. This calls for detailed knowledge on the olfactory and visual cues that parasitoids use to locate their hosts. To determine whether rearing methods can improve parasitoid efficacy, we investigated the effect of natal fly host species on host preference and parasitization behaviour of five pupal parasitoids, *Muscidifurax raptorellus* (Kogan and Legner), *Nasonia vitripennis* (Walker), *Urolepis rufipes* (Ashmead), *Trichomalopsis sarcophagae* (Gahan) and *Pachycrepoideus vindemmiae* (Rondani). Wasps were raised for either one or multiple generations on *Musca domestica* L. or *Calliphora vomitoria* L. (both Diptera) pupae and offered both fly hosts in a simple host choice set-up as well as a Y-tube choice assay. We chemically characterized the volatiles emitted by the pupae of these fly species and measured parasitoid responses following artificial transfer of semiochemicals between fly host species.

Key words: filth fly, pupal parasitoid, host preference, natal host, behavioural assays, semiochemicals, biological control

Olfactory circuits

Behavioral evolution in a complex world: From environment to neural circuits

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From dancing birds to singing flies, animals have evolved an astounding diversity of behaviors to attract and choose mating partners. Guided by innate preferences and aversions, the brain filters complex environments for cues and signals to successfully navigate these reproductive behaviors in appropriate spatial, temporal, and social contexts. To understand how the natural environment shapes sensory evolution and contributes to the diversification of neural circuits controlling mating behaviors, my lab studies the highly tractable nervous system of *Drosophila* as an inroad.

In *Drosophila*, courtship and mating occurs on fermenting food where many individuals congregate. Replicating this naturalistic context in the lab, we discovered striking differences in the sensory dependence of mating behaviors across species. Most notably, the host specialist *Drosophila erecta* requires the presence of food to engage in courtship and only mates in social groups. Harnessing the power of cross-species neurogenetics, we reveal that sexual arousal in *D. erecta* is uniquely gated by food odors that switch the valence of visual stimuli driving courtship. This provides a possible neural mechanism underlying the environmental modulation of reproductive behaviors, highlighting how social behaviors are shaped by the natural environments in which they evolve.

Olfactory circuits

Odorant receptor selectivity in ants

Author: Giacomo Glotzer¹

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Among insects, ants possess the largest odorant receptor (OR) repertoires, thought to relate to their reliance on pheromones to mediate colony communication. Yet we still lack a detailed understanding of how recently duplicated OR genes are chosen by neurons, integrated into circuits, and molded

by selection to drive behavior. Using the clonal raider ant *Ooceraea biroi*, a blind ant species that relies heavily on its olfactory system to mediate colony-level behavior, I investigate the development, wiring, and evolution of the ant olfactory system. At ESITO 2025, I aim to discuss a novel mechanism in the clonal raider ant that allows olfactory sensory neurons to produce tandemly arrayed ORs in a mutually exclusive manner. My results hint at a conserved strategy among hymenopteran insects and may help explain the rapid expansion and diversification of their OR repertoires.

Olfactory circuits

Hunger-state dependent olfactory processing in *Drosophila* larvae

Authors: Hari Pradeep Narayanan¹; Jonathan Ernst¹; Katrin Vogt¹

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Animals need to integrate external and internal information to inform navigational decisions during foraging. We showed that fly larvae switch from odor avoidance to odor attraction when food-deprived. This behavioral switch is mediated via increased serotonin release within the larval antennal lobe. We identified further that attractive odors can become more attractive in food-deprived larvae and that this enhancement in odor preference is also mediated via serotonin across different odors. We are investigating which specific serotonergic cell types are involved in the attractive enhancement phenotype. Within the antennal lobe, we screen for the requirement of olfactory processing neurons in the different behavioral states.

We have also established this enhancement phenotype in other innately attractive odours, corresponding to different olfactory receptors and receptor neurons. This suggests a potentially common mechanism which could have further implications given that the different odours signify distinct aspects of the larval environment.

In future experiments, we plan to compare the state-dependent activity levels of these cells in response to attractive odors, using a microfluidics device to immobilize fly larvae. Understanding the neural circuit plasticity underlying state-dependent processing across odors with different values in fly larvae will allow us to reveal general mechanisms that allow for flexible responses across phyla.

Olfactory circuits

Sexual pheromone processing in the honey bee brain

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To ensure reproduction, animals must efficiently detect and process intraspecific cues, such as sexual pheromones. In this study, we wonder how the brain of the honey bee, *Apis mellifera*, processes the unique sex pheromone described in this species, 9-oxo decanoic acid (9-ODA), which triggers male attraction to virgin queens during the mating. Previous work has shown that 9-ODA is detected by olfactory receptor AmelOR11, the most overexpressed receptor in male antennae compared to workers. We also know that this activates a specific, enlarged glomerulus in the antennal lobe, called macroglomerulus 2 (MG2). This neuronal pathway (AmelOR11 –MG2) is for now the only one described for the processing of 9-ODA within the honey bee male brain. To test whether this pathway is the only one responsible for male attraction to virgin queens, we generated AmelOR11 knock-out males using CRISPR-Cas9 genome-editing method. Mutant KO males showed an atrophied MG2 and

no response to 9-ODA in their antennal lobe using in vivo calcium imaging recordings. However, associative conditioning experiments showed that mutant males could still associate 9-ODA with a sucrose reward, revealing the capacity to detect 9-ODA through another pathway. The next step is to test the attraction of AmelOR11-KO males towards 9-ODA in a behavioural assay, using a four-way olfactometer, to determine whether this pathway is the only one involved in male attraction towards virgin queens.

Olfactory circuits

Dissecting odor coding strategies in the migratory locust

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² University of Wuerzburg

Understanding how odors are represented in the locust antennal lobe, which comprises over 2,000 glomeruli, has been a longstanding challenge. To investigate this phenomenon, we used the CRISPR-Cas9 system to generate transgenic migratory locusts (*Locusta migratoria*) that expressed the genetically encoded calcium indicator, GCaMP6f, in their olfactory sensory neurons. Using two-photon functional imaging, we examined the spatial activation patterns evoked by a variety of ecologically relevant odors. Our findings revealed a ring-shaped functional organization within the antennal lobe characterized by specific clusters of glomeruli. This spatial arrangement gives rise to an odor-specific chemotopic map in which distinct chemical classes and ecologically relevant odors are encoded as glomerular rings. Building on these results, we are currently investigating how these ring-shaped activity patterns differ between the migratory locust's two distinct phases: the gregarious and solitary states. These states exhibit pronounced ecological, behavioral, and phenotypic differences.

Olfactory circuits

Mechanosensory processing within the olfactory pathway

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While the role of the antennal lobe (AL) in processing olfactory signals is well-documented, its function in integrating multiple sensory modalities remains underexplored. In addition to olfactory processing there is increasing evidence in mammals and insects that mechanical stimuli are also processed within the olfactory pathway at an early processing level, indicating a close connection between both modalities. When we established multi-unit recordings in the AL of stick insects *C. morosus*, we observed the same phenomenon: subpopulations of the recorded AL projection neurons responded either to mechanosensory stimuli (14.4%), olfactory stimuli (11.9%), or both modalities (69.5%). Before investigating cross modal effects in neural coding within the AL, we aim to understand the features encoded within these mechanical responses. By applying acoustic vibrations with power spectra that differed primarily in three frequencies (265Hz, 600Hz and 900Hz), which we applied in three varying amplitudes, we analysed the effect of stimulus frequency and intensity. We found a variety of response patterns in individual AL-units that clearly depended on stimulus frequency presented. While some units showed an ordinary rate code, others revealed a combinatorial pattern of an excitatory response to one frequency and an inhibitory response to another. The latter is reminiscent of glomerular responses to mechanosensory stimuli within the AL of honeybees.

In addition to the frequency-dependent responses, we found an increasing response strength accompanying increasing stimulus amplitude, suggesting a loudness effect as well. Taken together, our results support the idea of parallel processing of olfactory and mechanosensory information within an early-processing neural circuitry in insects. Studying different aspects of these mechanosensory-olfactory interactions at various levels, from behaviour to neural processing, represents a necessary next step and will contribute to a comprehensive understanding of the insect olfactory system as a whole.

This work was funded by the DFG grant (STR 1334/4-1) to MSB as part of the research unit 5424 'modulation in olfaction'.

Odour-guided behaviour

Sensory flexibility in the desert locust - from social cues to collective migrations

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Locusts offer a powerful model for studying social plasticity and collective behavior. Adapted to extreme environmental fluctuations and population density shifts, they can rapidly transition from a solitary, sedentary phase to the notorious swarm-forming gregarious one. Despite their significance as a model system and the severe humanitarian impact of locust swarms, the mechanisms underlying their behavioral plasticity and the initiation of swarming remain poorly understood.

In this talk, I will discuss our work investigating the sensory basis of these transitions, focusing on the sensory representation of conspecifics and the local interaction rules that drive coordinated swarm movement. Our findings indicate that crowding enhances olfactory sensitivity, with gregarious locusts showing increased sensitivity to food cues in the presence of social ones—an adaptation that likely facilitates food detection in swarms. When moving between feeding sites, gregarious locusts move in cohesive groups where visual cues from conspecifics are both necessary and sufficient for coordinated movement. In this context, we found that while both solitary and gregarious locusts respond to visual motion cues, only gregarious individuals actively pursue them, suggesting a shift in visual processing that promotes collective motion.

Building on these findings, we currently work to further dissect the neural mechanisms mediating this transition, shedding light on how sensory processing reshapes behavior in different social contexts.

Odour-guided behaviour

Olfactory learning in *Pieris brassicae* butterflies is dependent on the intensity of a plant-derived oviposition cue

Authors: Joop van Loon^{None}; Marcel Dicke^{None}; Roos Lenders^{None}; Hans Smid^{None}; Dimitri Peftuloglu^{None}; Alexander Haverkamp^{None}; Stefan Bonestroo^{None}

Specialist herbivores, rely on specific cues to identify their host plants; however, these innate stimuli are often difficult to detect from a distance and herbivores might learn to associate additional olfactory or visual information with these cues. For many butterflies, oviposition occurs only when specific gustatory cues are detected by the tarsi, allowing a mated female to identify ideal sites for offspring development. However, upon substrate location, simultaneously occurring stimuli might be integrated in memory and used in a later moment as additional cues to locate oviposition sites. In the present study, we asked whether mated *Pieris brassicae* butterflies are able to associate a novel olfactory stimulus, vanilla scent, with an innately meaningful oviposition stimulus, the glucosinolate

sinigrin. In addition to this, we asked whether memory recall was dependent on the intensity of the neuronal response generated by sinigrin tarsal detection. In a first moment, mated butterflies were conditioned by exposure to a paper disc sprayed with different sinigrin concentrations and coupled with a vanilla scent source. To assess memory formation, animals were later exposed to the same paper discs used during conditioning, either bearing vanilla scent or a control solvent, but not sprayed with sinigrin. Memory recall was assessed by measuring 1) time spent on each disc type, 2) number of visits to each disc type, 3) number of oviposition events on each disc type. Tests were performed indoors (greenhouse) and outdoors (flight tent) and in both cases butterflies were able to form memory after conditioning. Moreover, electrophysiological recordings on the tarsal taste sensilla used to detect sinigrin allowed to observe that memory recall occurred only for animals conditioned with sinigrin concentrations giving the highest neuronal responses. This study presents novel insights on how butterflies can integrate different sensorial information to successfully complete crucial tasks such as oviposition, showing how cognitive plasticity might contribute to adaptation in a rapidly changing environment.

Odour-guided behaviour

YOLOto: an open-source mosquito detector for behavioral tracking

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Mosquito-borne diseases pose a major global health burden. Effective vector control depends on understanding how mosquitoes interact with their environment, making behavioral research essential. Object detection models can be trained to distinguish mosquitoes from complex backgrounds, offering high-throughput solutions for behavioral tracking. Here, we present YOLOto (YOLO-Mosquito), an open-source detector based on YOLOv11, fine-tuned specifically for mosquito detection in both laboratory and real-world settings. Trained on over 100,000 annotated images from public datasets and research labs worldwide, YOLOto detects multiple mosquito species under diverse conditions and achieves high performance on an unseen test dataset. In addition, we release a ready-to-use analysis toolkit that enables quantification of trajectory coordinates, visit frequency, duration, and distance. We also demonstrate its capabilities in a case study involving host-seeking female mosquitoes. YOLOto provides a fast, accurate, and cost-effective tool for advancing behavior-based mosquito research and can improve vector control strategies.

Odour-guided behaviour

Olfactory navigation in the Australian Bogong moth *Agrotis infusa*

Authors: Christer Löfstedt¹; Eric Warrant¹; Hong-lei Wang¹; Hui Chen¹; Linnea Rosberg¹

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The Bogong moth *Agrotis infusa* is a long-distance nocturnal migrant whose navigational ability still remains only partly understood. They travel up to a thousand kilometres to the Australian Alps

where they seek out the shelter of isolated cool alpine caves, thereby escaping the approaching summer heat of their breeding grounds. Once in the caves, they enter a state of dormancy over the coming summer (known as aestivation). How they find their aestivation caves is still a mystery. Our hypothesis is that at the end of their journey, during the final “pin-pointing phase” of their long-distance navigation, they use their olfactory system to home in on their cave destination. If this is true it would possibly be the first proof of an innate “homing” olfactory cue.

We investigated the olfactory system of the Bogong moth to address this hypothesis by using odour collections from the caves (analysed in a gas chromatograph-mass spectrometer), electrophysiological recordings from the antennae and behavioural experiments. When analysing cave odours, one compound - (Z,E)-9,12-tetradecadienyl acetate - was found that males strongly react to electrophysiologically and both sexes behaviourally. During the spring migration towards the caves they are significantly attracted to this compound, but when leaving the caves on their return journey in the autumn, they are indifferent to it. The origin of this compound remains unknown. However, the three likeliest sources are the moths themselves, microbial activity in the soil or one or both species of parasitic mermithid nematodes that live only in these caves.

Odour-guided behaviour

Risk assessment in a navigating desert ant

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The Tunisian desert ant *Cataglyphis fortis* inhabits the hostile salt pans where it forages for dead arthropods. Some years ago we found that homing foragers locate their nest entrance by following a plume of carbon dioxide emanating from the nest entrance (Buehlmann et al. *Current Biology* 2012). Since this plume is not nest specific and ants are inevitably killed when they enter an alien nest, homing foragers only follow a plume when path integration, their main navigational tool, tells them that they are close to home. In addition, we have recently found that many foragers, although well adapted to their harsh environment, die from heat stress during foraging trips (Freire et al *Current Biology* 2023). Especially when returning from very long foraging trips, path integration is often not accurate enough to guide the ants directly back to the nest. The ants then begin a systematic search, which increases their chances of finding the nest entrance, but also exposes them further to the desert heat. Here we show that ants make a risk assessment during their search between being killed by the sun and being killed by an alien nest. Ants that have searched their nest for a while give up on safety and follow nest plumes even when the path integrator tells them that they may be far from home.

Odour-guided behaviour

Host plant selection and discrimination of mosquitoes

Author: Rickard Ignell¹

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Anthropophilic mosquito species depend on plant-derived sugars for building energy reserves for flight and survival, as well as reproduction, and is thus an established fact. There are, however, still a large number of open questions to be addressed, such as the natural range of host plant species and how this is affected or affects tritrophic interactions with pathogens, such as the malaria parasite. An insight into the chemical basis of

how mosquitoes select and discriminate among potential host plants may be of general interest for those studying insect-plant interactions, and may have implications for the development of novel attractants used in attractive toxic sugar bait technology. In my presentation, I will provide an insight into what my lab has working on, in relation to these aspects.

Odour-guided behaviour

Functional characterization of ovipositor-expressed odorant receptors in *Spodoptera littoralis*

Authors: Sai Zhang¹; Arthur Comte¹; Nicolas Montagné¹; Guirong Wang²; Emmanuelle Jacquin-Joly¹

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Odorant receptors (ORs) are key components of the insect olfactory system and are primarily expressed in antennae, where they mediate the detection of environmental volatiles. Interestingly, certain ORs have also been identified in non-antennal tissues like the ovipositor, suggesting possible roles in regulating oviposition behavior. In this study, we performed transcriptomic analyses across different tissues of the noctuid moth *Spodoptera littoralis* and identified four ORs expressed in the ovipositor. Using structure-based virtual screening (SBVR) combined with experimental validation, we could identify ligands for three out of the four ORs. Oviposition behavioral assays revealed that four out of the six optimal ligands exhibited significant oviposition deterrence in female moths. To determine whether the ovipositor plays a role in detecting these cues, antennal ablation was conducted prior to oviposition assays. The deterrent effects were largely abolished, except for indole, suggesting that ovipositor may contribute to its detection. We subsequently utilized a four-arm olfactometer to investigate whether these ligands elicit orientation responses in moths. The results showed that all tested compounds elicited significant repellent responses. Notably, indole continued to induce avoidance behavior after antennal ablation, supporting a potential role of the ovipositor in its detection. Collectively, these results provide new insights into the molecular and behavioral mechanisms of oviposition site selection and expand our understanding of peripheral olfactory mechanisms in moths.

Pollination ecology

How biotic and abiotic factors impact the evolution of plant-insect interactions

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Both biotic (e.g. pollinators, herbivores) and abiotic ecological factors (e.g. soil, temperature) cause selection and thus impact adaptive evolution in plants. Nevertheless, we know little about the impact of individual factors and their interactions, as well as the speed of evolutionary adaptation, because of the paucity of controlled experimental studies addressing these questions in plants and insects. Since several years my group has been using experimental evolution in semi-natural conditions in the greenhouse, to study real-time plant evolution driven by different ecological factors. In my talk I will give an overview of some of the evolutionary effects that we have demonstrated, with particular focus on floral attractiveness- and defense traits. We have found that efficient generalist bee pollinators like bumblebees select for taller plants and more attractive flowers, including higher scent emission and UV reflection. Low efficient pollinators on the other hand select for more selfing. Bumblebees also select against defense compounds, whereas herbivores select for more defense, leading to a reduction in the attractiveness of flowers. For the abiotic factors, soil enables or disables evolutionary responses to selection, through the availability of nutrients. Increased temperature leads to reduction in floral volatiles, and more but smaller flowers. Biotic and abiotic factors also show strong interactive effects, for example, bumblebee pollination leads to phenotypic divergence in response to soil type, and elevated temperature leads to more UV-reflection with bumblebee pollination. Overall, experimental evolution shows how plants can rapidly adapt to environmental factors, explaining the geographic variability in plant traits in nature. Our experiments also show how environmental change may impact evolutionary trajectories of plants in the future.

Pollination ecology

The role of floral scents composition in plant-pollinator interactions

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Floral scents function as both long- and short-distance cues, conveying information about a plant's presence, identity, and resource availability. The success of plant-pollinator interactions depends critically on pollinators' responses to these volatile organic compounds (VOCs). A central feature of floral scent is its evolutionary lability: in this tight retroaction cycle, emission of and response to floral volatile organic compounds (VOCs) are the first line of change. Thus, the composition of floral scent bouquets could potentially serve as an indicator of the pollinators with which the plants interact the most.

Traditionally, studies on VOC-mediated plant-pollinator networks classify pollinators by pollination syndrome and VOCs by biosynthetic pathway. However, recent findings demonstrate that even a single compound difference between two closely related plant species can account for the difference in behavioral response of two closely related pollinator species. Moreover, phylogenetically close plant species inhabiting different ecological communities may exhibit divergent scent profiles that converge with co-flowering species sharing the same pollinators. This suggests that floral VOC composition may reflect ecological interactions more than phylogenetic history.

In this study, we aim to identify specific VOCs—or functionally relevant groups—that structure plant-pollinator interaction patterns, without relying on predefined categories for pollinators, plants, or compounds. We compiled data on floral scent composition and plant-pollinator interactions from published literature and databases. Using network-based clustering methods, we grouped pollinators and VOCs based on shared interaction patterns, enabling us to explore whether pollinator visitation is associated with particular scent profiles.

Our approach provides a data-driven framework to uncover the role of chemical signatures and pollinator olfactory sensory bias in pollination networks, with implications for community ecology, conservation of pollinators, and optimization of pollination services in agroecosystems.

Evolution

Chemical sensing in arachnids

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Arthropods exhibit a wide array of chemical senses that are essential for survival, reproduction, and environmental interactions. Most of our understanding of arthropod chemical senses is derived from research on insects, whose chemosensory systems are notably advanced. The molecular foundation of these systems involves specialized proteins encoded by extensive gene families that have experienced considerable expansion and diversification throughout insect evolution. We also have some knowledge regarding the chemical senses of crustaceans, with a primary focus on decapod crustaceans. However, a comprehensive understanding of the evolution of chemical senses in arthropods requires insights into myriapods and arachnids, which remain largely unexplored. Given that terrestrial adaptation occurred independently multiple times in arthropods, it is plausible that olfactory sensilla also evolved several times independently. I aim to summarize the current knowledge on chemosensilla in arachnids and propose a framework that may facilitate the formulation of specific research questions and identification of target groups for further study.

Evolution

Where's Waldo? A hunt for functionally divergent odorant receptor orthologs in the context of host shifts

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Olfactory adaptations play a key role in initiating or stabilizing dietary shifts by reshaping the insect's responsiveness to host-specific chemical cues. Modulating gene expression or altering the specificity and sensitivity of odorant receptors (ORs) can influence an insect's ability to recognize host volatiles. However, highlighting these specific molecular changes remains a challenge. Insects can possess hundreds of ORs, most of which are still orphan receptors with unknown ligands. This limited functional characterization constrains current efforts to identify functionally divergent orthologs, which typically rely on sequence similarity alone.

Structure-based virtual screening (SBVS), which employs molecular docking simulations, offers a promising solution to overcome this limitation. While widely used for predicting ligand–receptor interactions in mammalian G protein-coupled receptors (GPCRs), SBVS has only recently been adapted to insect ORs. This strategy allows rapid, repertoire-wide prediction of odorant binding profiles, facilitating cross-species comparisons of olfactory tuning and the identification of functionally divergent receptors associated with ecological specialization.

In this study, we present the first application of SBVS to the entire OR repertoire of an insect, leveraging this approach to explore peripheral olfactory system adaptation associated with host plant shift in the genus Spodoptera. We annotated the full OR repertoire of the oligophagous species *S. picta*, which feeds exclusively on Amaryllidaceae, and compared its predicted chemical detection space to that of its two polyphagous sister species, *S. litura* and *S. littoralis*. We complemented this approach with RNA sequencing to determine the expression profiles of SpicORs. This integrative approach led to the identification of SpicOR29, a receptor highly expressed in *S. picta* that exhibited distinct sensitivity and selectivity toward volatiles emitted by its Amaryllidaceae hosts (*Clivia miniata* and *Crinum* spp.), in contrast to its orthologs in the polyphagous species. Site-directed mutagenesis revealed a single amino acid substitution in SpicOR29 as a key determinant of its shifted ligand sensitivity.

Evolution

Olfactory alchemy: Unraveling the molecular basis of the Orco-ORx puzzle

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Olfaction is one of the most ancient sensory modalities that insects rely on both for survival and to interact with the environment. Insects use different types of receptors to detect airborne chemicals, one of them being olfactory receptors (ORs). In winged insects, a functioning OR signaling complex is likely a heterotetramer comprising two different types of subunits: the conserved co-receptor, Orco, and a tuning receptor ORx subunit which provides odorant sensitivity. Recent discoveries

shed light on the structural organization of Orco and ORs revealing the key interacting domain between different subunits that form this homomultimeric receptor and the ligand binding site. The location of the ligand binding pocket and the interacting amino acid residues that are responsible for the sensitivity of the receptor are known. However, understanding of the molecular intricacies governing the formation and assembly of functional complexes between the Orco and ORs remains elusive. Our research aims to address this gap by combining phylogenetic analysis of naturally occurring and divergent Orcos, complex modeling, and functional recordings. Specifically, we are elucidating how a highly conserved Orco assembles and forms functional complexes with multiple divergent ORs. By comparing interactions between Orco and a variety of differently tuned ORs, we also aim to identify differential mechanisms underlying complex formation. Overall, this research will deepen our understanding of the precise molecular interactions between the tryptic formed by Orco-ORx complexes and their ligands.

Evolution

Of flies and feces: Olfactory preference for weird resources in *Drosophila melanogaster*

Authors: Heinrich Marx¹; Markus Knaden¹

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The genus *Drosophila* comprises numerous species that differ in their microhabitats, as well as in their olfactory preferences. Here, we conducted artificial selection experiments to induce olfactory preference for novel food sources in *Drosophila melanogaster*. In two-choice experiments, the flies could choose between an odor they are usually attracted to, such as the odor of yeast, and the odor of human or horse feces. Although feces are an energy-rich, abundant resource utilized by various organisms, including Diptera,

D. melanogaster does not feed on or reproduce in feces. The few flies that chose feces were bred further, and their offspring's preferences were tested each generation. Regardless of whether the experiment was conducted with the Canton-S laboratory strain or a line of wild-caught flies, we found a significantly increased preference for feces odor after eight generations.

In a follow-up study, we will investigate whether this change in preference is accompanied by increased sensitivity to feces odors and/or decreased sensitivity to yeast odors.

Evolution

Evolution of an olfactory subsystem in Hymenoptera: a springboard for eusociality?

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The Hymenoptera present the highest number of eusocial species in insects and up to 9 independent origins of eusociality. By contrast, the eusocial lifestyle is virtually absent in other taxa, suggesting the existence of facilitating factors leading to advanced social organisation within Hymenoptera. As eusocial evolution is explained by the concept of inclusive fitness and the theory of kin selection, an ability to distinguish kin from non-kin could be instrumental for the advent of higher levels of sociality, by preventing costly altruistic acts towards unrelated individuals. Recent studies suggest

that eusocial ants possess an olfactory subsystem that is specialised in the detection of cuticular hydrocarbons, providing a potential mechanism to detect social identity. It involves a particular type of sensilla on the antenna, the basiconic sensilla. They house olfactory sensory neurons (OSN) that are thought to express a group of odorant receptor genes with a distinctive 9-exon structure. These OSNs project to a segregated region of the primary olfactory centre in the brain, the antennal lobe. Strikingly, a similar suite of features has been observed in Vespidae wasps which independently evolved a eusocial lifestyle, suggesting a potential link between this olfactory subsystem and advanced forms of social organization. By combining broad taxonomic sampling of Hymenoptera species with detailed neuroanatomical, functional and molecular characterisation of their olfactory structures, we aim to address the potential role of the basiconic-sensilla subsystem in social evolution.

Evolution

Comparative transcriptomic analyses in Tephritidae species provide insights into the ecological driving force behind odorant receptor evolution

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The insect olfactory system has evolved to guide species toward specific mating partners, food sources, and oviposition sites. However, how species-specific repertoires of odorant receptors (ORs)—which mediate the detection of volatile cues—have been shaped by ecological pressures remains poorly understood. Fruit flies of the Tephritidae family (Diptera) exhibit highly diverse host preferences due to multiple host switches throughout their evolutionary history, making them ideal models to explore this question.

In this study, we used RNA sequencing to identify the OR repertoires of thirteen Tephritidae species with varying host ranges: *Bactrocera curvipennis*, *Bactrocera dorsalis*, *Bactrocera psidii*, *Bactrocera tryoni*, *Bactrocera umbrosa*, *Bactrocera zonata*, *Ceratitis capitata*, *Ceratitis catoirii*, *Ceratitis quilicii*, *Dacus ciliatus*, *Dacus demmerezi*, *Neoceratitis cyanescens*, and *Zeugodacus cucurbitae*. Through manual curation, we annotated 60 to 80 OR transcripts per species, including the obligatory coreceptor Orco, and identified a total of 698 new OR sequences. Differential expression analyses revealed some organ- and sex-biased OR expression patterns. Importantly, we found significant correlations between certain OR repertoire characteristics and host range. Specifically, both the sequence and relative expression levels of several ORs were more conserved in polyphagous than in oligophagous species. Additionally, certain ORs were uniquely present in polyphagous species. These findings provide molecular insights into the ecological forces shaping OR evolution in Tephritidae fruit flies.

Evolution

Receptor expression analysis of an olfactory organ reveals evolutionary patterns: from co-option to cell-type diversification.

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Understanding how sensory organs evolve is a central question in sensory biology and evolutionary neuroscience. Due to their complexity, comprehensive analysis of entire sensory organs has been challenging. Here, we leverage the relative simplicity of the *Drosophilid* maxillary palps: olfactory organs critical for odor-guided behavior and host-seeking. In *Drosophila melanogaster*, these organs comprise only six neuronal types organized into three stereotypical sensillum types, offering a tractable system for evolutionary analysis.

Using a combination of cross-species transcriptomics, receptor expression profiling, and genetic tools, we uncover fundamental mechanisms driving olfactory organ evolution. Our findings reveal widespread co-option of olfactory receptors across organs and life stages. We dissect the genetic basis of evolutionary shift and identify key changes in cis-regulatory elements in one of these receptors. Importantly, we identify a specific neuronal cell type that consistently serves as a hotspot for evolutionary changes in receptor expression across species. Furthermore, by examining species spanning broad evolutionary distances, we identify the emergence of novel neuron types, characterize evolutionary intermediates, and show that while receptor expression is highly adaptable, the evolution of new cell types is more constrained.

Altogether, our work provides new insight into how sensory organs evolve, highlighting both conserved and adaptable components of olfactory system architecture.

Evolution

From land to water: Evolution of odorant receptor repertoires and expression across aedeophagan beetles

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The suborder Aedeophaga, second suborder of Coleoptera in species number, includes families from a wide range of ecological environments, from terrestrial to aquatic. Multiple independent transitions from land to water within this group provide a valuable model for studying how the olfactory system adapted to different environments. Given the distinct properties of chemical diffusion in air versus water, chemosensory evolution is expected to reflect major morphological and molecular shifts. Our work investigates the evolution of the chemosensory system in aquatic Aedeophagan-beetles, using a transcriptomic approach. First, a total of 24 transcriptomes were sequenced, assembled, annotated, and their expression were quantified among antennae and palps from species representing seven Aedeophagan families, including both terrestrial and aquatic species. Secondly, a case study on *Cybister lateralmarginalis* (Dytiscidae) involved comparing gene expression across different cephalic organs in both larval and adult stages.

Taken together, our results show that in terrestrial Aedeophaga, odorant receptors (ORs) are predominantly expressed in the antennae, with no evidence of ORs exclusively expressed in the maxillary palps. In contrast, aquatic species exhibit clades of ORs specifically expressed in the maxillary palps. Notably, two of these clades show substantial gene duplications (expansions) that are specific to aquatic families. We hypothesize that these maxillary-palp-specific ORs are localized to a lateral olfactory field on the palp, as supported by scanning electron microscopy (SEM) and in situ hybridization targeting the co-receptor Orco. In Dytiscidae larva, we also identified a set of larval-specific ORs that are not expressed in adult, suggesting stage-specific olfactory functions and further diversification of the chemosensory system across developmental stages. These results highlight a striking

divergence in the olfactory system between terrestrial and aquatic Adephaga, with evidence for molecular innovations pointing to a major evolutionary shift in chemosensory strategies associated with habitat transition.

Evolution

Evolutionary reconstruction of odorant receptor co-expression, neofunctionalization, and replacement

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The cell-type specific pattern of odorant receptor (*Or*) expression, paired with their high rates of duplication, raises the question: *How are the genetic origins of new Ors “coordinated” with the development of olfactory sensory neurons (OSN) so that they are uniquely expressed?* To answer this question, we need to identify newly evolved *Or* genes and investigate how they have acquired their OSN-specific expression. We use the most duplicated/deleted *Or* subfamily in the *D. melanogaster* species group - the *Or67a* subfamily - as our experimental system. Our evolutionary analyses revealed that this subfamily is notable not only for its extensive copy number but also for containing jumping genes that have recurrently seeded new *Or67a* members over the flies' main chromosomes. Several *Or67a* duplicates have experienced adaptive protein changes, with *in vivo* electrophysiological recordings substantiating their quick functional diversification. Contrary to the standard “one receptor-one neuron” expression pattern, we found that most *Or67a* duplicates are co-expressed with one or more *Or67a* copies in the same OSN population. However, despite co-expression being the dominant pattern, we also identified two instances of *Or67a* duplicates that have “escaped” co-expression and evolved distinct OSN expression. Using a combination of *in situ* hybridization and single nuclei RNA-seq experiments in *D. suzukii* and closely-related species, we discovered that these two *Or67a* copies have gained expression in preexisting neuron populations, having replaced their previous *Ors*. Our data suggest a model of *Or*-OSN evolution in which co-expression is often the first step of peripheral diversification. While co-expression may occasionally be adaptive, we suspect that it is frequently be transient, reflecting the contingent and opportunistic nature of evolutionary change. Our results also suggest that *Or* replacement outpaces the evolution of OSN, highlighting limitations in defining these cell-types based on their receptors alone.

Gustation

Evolution of taste processing shifts dietary preference

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Food choice is an important driver of speciation and invasion of novel ecological niches. However, we know little about the mechanisms leading to changes in dietary preference. We use the three closely-related species *Drosophila sechellia*, *D. simulans* and *D. melanogaster* to study taste circuit and food choice evolution. *D. sechellia*, a host specialist, feeds exclusively on a single fruit (*Morinda citrifolia*, noni) - the latter two are generalists living on diverse diets. Using quantitative feeding assays, we recapitulate the preference for noni in *D. sechellia* and detect conserved sweet but altered bitter sensitivity via calcium imaging in peripheral taste neurons. Noni surprisingly activates bitter

sensing neurons more strongly in *D. sechellia* due to a small deletion in one single gustatory receptor. Using volumetric calcium imaging in the ventral brain, we show that instead of peripheral physiology, species-specific processing of noni and sugar signals in sensorimotor circuits recapitulates differences in dietary preference. Our data support that peripheral receptor changes alone cannot explain altered food choice but rather modifications in how sensory information is transformed into feeding motor commands.

Gustation

Neuroethology of taste in the honey bee *Apis mellifera*: from behaviour to the neural circuits

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The honey bee *Apis mellifera* is a traditional model for the study of olfactory perception, information processing, and learning abilities. Surprisingly, this species' sense of taste remains largely understudied compared to the other sensory modalities. Nonetheless, honey bees' gustatory abilities are crucial for the survival of the species, since they influence food choices and enable the avoidance of toxic substances. My project seeks to fill the gaps in the study of honey bee gustation by exploring the mechanisms underlying taste information processing through two complementary approaches: behaviour and neuroanatomy. First, through the adaptation of the Multi-Capillary Feeder assay (MultiCaFe) on the honey bee, we evaluated the bees' ability to detect sixteen tastants belonging to three gustatory qualities (salts, bitters, and amino acids), each tested at six different concentrations. This allowed the inference of *A. mellifera*'s gustatory preferences and the classification of the stimuli as phagostimulants or repellents for this species. Second, we developed a new set-up to perform appetitive associative conditioning experiments, in which bees associate gustatory stimuli with a sucrose reward. This allowed us to demonstrate that bees are able to discriminate between stimuli of the same hedonic value, despite having a limited number of taste receptors (11 GRs). Finally, we performed a neuroanatomical study to describe the gustatory pathways in the honey bee brain by means of fluorescent tracer injections, both in the taste appendages (proboscis, antennae, and tarsi) and in the central brain. We showed that the spatial organization of gustatory appendages is centrally reflected in the distribution of gustatory afferents. The overlap of projections is notable among inputs from the same appendage origin (e.g., between mouthparts) and remains limited between those from different origins (e.g., antennae and mouthparts).

Modulation and Plasticity

The ecological mind —making sense of the world with a tiny brain

Author: Sophie Caron¹

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Brains are evolved to learn, enabling animals to respond flexibly to an unpredictable world and to draw on experience to guide future behaviors. To learn efficiently, brains need to balance a high degree of flexibility in the representation of the outside world with the limited neuronal infrastructure available to them. Focusing on the *Drosophila melanogaster* olfactory system, my research investigates how the neuronal circuits that enable efficient learning are pre-configured to interpret

the world. In my presentation, I will explore two key questions: First, what are the neural pre-configurations that allow the brain to learn efficiently from the environment? Second, how are these pre-configurations shaped by the ecological conditions in which brains evolve? Using a wide range of approaches —from neurogenetics and behavioral assays to cross-species comparisons and the study of species interactions —we reveal the subtle ways in which ecology shapes neuronal circuit architecture and function.

Modulation and Plasticity

Olfactory-visual integration and recurrent modulation of olfactory processing in the honeybee

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The European honeybee, *Apis mellifera*, is a favourable experimental model for investigating cross-modal sensory interactions, particularly olfactory-visual integration, at both the behavioural and neuronal levels. Such integration occurs in high-order processing centres called mushroom bodies (MB), which receive sensory information from primary olfactory and visual centres, the antennal and optic lobes (AL and OL, respectively). Axon terminals of projection neurons from the AL and OL form synaptic complexes with MB intrinsic neurons, the Kenyon cells, in the MB calyx. The information is then transferred via Kenyon cell axons to the MB output regions, the medial and vertical lobes. Here, Kenyon cell axons converge synaptically with MB output neurons (MBONs), which form short-range recurrent connections to the MB calyx and centrifugal long-range connections to the AL, lateral horn, and other regions of the protocerebrum. It has been shown that individual MBONs respond to odour or light stimuli, and a substantial proportion is sensitive to both odour and light stimuli (Strube-Bloss & Rössler 2018, Roy Soc Open Sci). This shows that multimodal convergence is present at the MB output level. Are differential responses to the two sensory modalities represented in morphologically distinct MBON clusters? How is cross-modal information distributed to upstream and downstream olfactory processing centres? We perform electrophysiological intracellular recordings from MBONs in the MB vertical lobe while presenting adult honeybees with an olfactory-visual stimulation protocol to physiologically characterize individual MBONs. We combine this with iontophoretic current injections and fluorescent labelling techniques to identify the anatomical convergences of individual MBONs with the AL, lateral horn and other parts of the protocerebrum. We use a recently established detailed 3D atlas of the honeybee brain (Habenstein et al. 2023, J Comp Neurol) as a reference for mapping target innervation patterns of MBONs in upstream and downstream olfactory neuropils. We show cases of MBONs responding to light stimuli that provide centrifugal input to olfactory centres, such as the AL, indicating long-range feedback modulation of olfaction via visual input. Ultimately, our studies aim to better understand cross-modal interactions underlying context- and experience-related modulation of olfactory processing and olfactory-visual perception.

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Modulation and Plasticity

Nutrient specific modulation of sensorimotor processing in *Drosophila melanogaster*

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Animals adapt their behavior to meet changing internal needs, such as the requirement for specific nutrients to maintain homeostasis. While it is known that internal states like nutrient deprivation or reproductive status drive appetite for foods that are rich in particular nutrients, the neural mechanisms by which these needs shape behavior remain incompletely understood.

Building on our previous work that established a functional atlas of the subesophageal zone (SEZ) and identified SEZ regions involved in protein appetite, we now focus on how carbohydrate deprivation alters sensory processing. Using volumetric 2 photon imaging, behavior, and genetic manipulation, we show that carbohydrate deprivation engages distinct SEZ regions, separate from those modulated by protein hunger. Furthermore, we identify candidate driver lines and neurons that innervate these carbohydrate sensitive regions and contribute to carbohydrate specific appetite. These findings indicate that nutrient specific deficits activate separable neural pathways to flexibly adjust sensorimotor processing based on internal need, highlighting how the brain dynamically adapts sensory processing to guide nutrient specific food choice and maintain physiological balance.

Modulation and Plasticity

Hunger vs. hazard: Neurophysiological mechanisms of maladaptive food intake

Author: Dennis Pauls^{None}

Hunger or malnutrition results in insufficient caloric intake, triggering profound physiological changes that can culminate in organ damage or death. To prevent this, organisms may engage in maladaptive food intake, consuming food sources that are normally avoided due to their poor quality or potential harm. However, the underlying neurophysiological and metabolic mechanisms that drive such a survival-orientated, yet risky feeding behaviour remain poorly understood. Our project investigates how brain-body communication shifts to promote maladaptive food intake under nutrient stress. Using *Drosophila*, we aim to characterize the metabolic signatures, endocrine factors, and neurophysiological pathways that enable this context-dependent regulation of feeding behaviour. Our findings identify adipokinetic hormone as a metabolic trigger that, via octopamine-mediated neuromodulation, alters brain body communication to promote maladaptive food intake and thereby compensate for energy deficits.

Modulation and Plasticity

Target- and type-specific synaptic plasticity of olfactory sensory neurons

Author: Robert J. Kittel¹

¹ *GNOI*

Specific olfactory receptors tune the sensitivity of chemoreception, enabling olfactory sensory neurons (OSNs) to detect odours across a broad spectrum of intensities. In order to study additional mechanisms of odour-specific neuronal processing, we investigated OSN synapses in the *Drosophila* antennal lobe, the first relay station of the olfactory pathway. Here, we examined the structure and function of presynaptic active zones (AZs), where specialised molecular complexes control neurotransmission. Artificially reducing neurotransmitter release probability of OSN AZs disrupts both neural coding and behavioural reliability. Strikingly however, target-specific synaptic plasticity rescues these defects within a day. This homeostatic compensation is mediated by AZ biogenesis and requires the secretory pathway protein Sec31. Furthermore, our data show that highly sensitive OSNs expressing the alarm-odour receptor Or56a have a limited capacity for homeostatic plasticity and display a different layout of core AZ proteins compared to conventional food-odour detecting OSNs. We hypothesise that these features reflect the basal tuning of Or56a OSNs towards maximum levels of performance.

Modulation and Plasticity

Sensory cross-talk between sight and smell in higher order brain centers

Authors: Devasena Thiagarajan¹; Veit Grabe²; Silke Sachse¹

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In nature, insects perceive and consolidate complex multisensory cues to elicit diverse behaviors. Our previous work using a classical conditioning paradigm shows that *Drosophila melanogaster* benefits from bimodal sensory integration when associating punishment signals with weakly learned olfactory and visual cues on a need-to basis. By adapting our behavioral paradigm to a physiological setup with a two-photon microscope, we performed bimodal aversive conditioning on a living fly while recording calcium-dependent neuronal responses from select populations in higher-order brain centers, such as the mushroom body (MB) and lateral horn (LH). Consistent with our behavioral results, we observed differential modulation in the responses of these neurons to odor and light stimuli after unimodal and bimodal training, revealing a potential multimodal integration site that consolidates contextual information mediated by the LH and associative learning information mediated by the MB. Further experiments inhibiting the functionality of these neurons could clarify their role in bimodal sensory integration and provide insight into the fundamental neuronal mechanisms underlying multisensory processes.

Receptor function

Structural mechanisms of chemical specificity in insect odorant receptors

Authors: Steve Jang^{None}; Sanjana Mandala^{None}; Josefina del Marmol^{None}

A large diversity of odorant molecules reaches the antennae of insects at any given time. The key role of odorant receptors (ORs) is to translate the chemical complexity of the environment into interpretable neuronal signals. To this goal, ORs exhibit a wide range of ligand specificities: many ORs are promiscuous, responding to dozens of chemically diverse odorants, and some ORs are exquisitely selective towards just one chemical cue. In this talk, I will present our work towards understanding how ORs achieve diverse chemical specificity. Using cryogenic electron microscopy (cryoEM), electrophysiology and comparative studies, we characterized ORs from various insect species, shedding light on the diverse structural strategies by which odorant receptors achieve both promiscuous and selective odorant specificity.

Receptor function

Identification of two odorant receptors tuned to alarm pheromone in the honey bee *Apis mellifera*

Authors: Alizée Delarue¹; Benjamin Andreu¹; Emmanuelle Jacquin-Joly²; Jean-Christophe Sandoz^{None}; Julia Mariette¹; Julie Carcaud¹; Nicolas Montagné²; Thomas Chertemps²; Virginie Larcher¹

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The success of social insect colonies lies in the capacity of all members of the society to behave in a well-organized and context-dependent manner, thanks to elaborate communication among

colony members. Honey bees, in particular, use a sophisticated chemical communication system based on the use of a high number of pheromones, most of which have already been identified. How does the social insect brain manage to encode such a plethora of highly-meaningful and ecologically-relevant signals? Despite substantial knowledge acquired on olfactory processing in this species, the mechanism of pheromonal coding remains poorly understood. In particular, olfactory receptors (ORs) detecting social pheromones are still unknown. We thus used heterologous expression in the *Drosophila* “empty neuron system”, coupled with transcuticular calcium imaging and electrophysiology. We deorphanized two odorant receptors, AmelOR136 and AmelOR109, which detect constituents of the alarm pheromone. AmelOR136 exhibits a sparse coding strategy, suggesting a finely tuned mechanism for efficient communication in alarm situations. In contrast, AmelOR109 is a more broadly-tuned receptor, responding to diverse odorants, including pheromones. This study paves the way for understanding pheromonal coding in the honey bee brain.

Receptor function

Unravelling pheromone detection in the Asian and American palm weevils

Authors: Ludvine Brajon¹; Arthur Comte²; Emmanuelle Jacquin-Joly³; Nicolas Montagné¹

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The red palm weevil (*Rhynchophorus ferrugineus* –RPW) and the American palm weevil (*Rhynchophorus palmarum* –APW) are major pests that cause significant economic losses to palm crops. These two species use aggregation pheromones to gather for feeding and mating on their host plants, specifically palm trees. Despite their close phylogenetic relationship, RPW and APW are allopatric species that rely on distinct aggregation pheromones: a mix of ferrugineol/ferruginone and rhynchophorol, respectively. To date, only one pheromone receptor, RferOR1, has been characterized in RPW, leaving the molecular basis of pheromone detection unexplored in these two species.

Here, we expand on this knowledge by identifying several additional pheromone receptors in these species. Phylogenetic analyses reveal that these newly identified receptors belong to distinct evolutionary lineages, suggesting multiple independent events of pheromone receptor emergence. To better understand how these receptors recognize their respective pheromone compounds, we employed molecular docking simulations. These *in silico* approaches provided valuable insights into the structural mechanisms underlying pheromonal recognition and the evolution of pheromone specificity in *Rhynchophorus*.

Our findings contribute to improve our understanding of pheromone detection in these pests, and establish the foundation for the development of innovative targeted control strategies based on disrupting pheromone receptor functioning.

Receptor function

From the depths of the twilight zone: expression & functional analyses of an odorant & gustatory receptor homolog conserved in flies, humans and beyond

Authors: Nathaniel Himmel¹; Liliane Abuin¹; Leana Keel¹; Florian Fournes¹; Justine Collier¹; Richard Benton¹

¹ *University of Lausanne*

Using protein structure modeling-based methods, we recently identified very distant insect Or/Gr homologs, to define a protein superfamily we named 7-Transmembrane domain Ion Channels (7TMICs). 7TMICs are present across the tree of life and likely originated in the Last Universal Common Ancestor (~4 billion years ago). 7TMICs can be classified into two families: Class-A, which includes the insect Ors/Gr and a large family of plant receptors, among many others, and Class-B, functionally mysterious proteins present in invertebrates and vertebrates, including humans, whose common ancestor with Ors/Gr existed ~2 billion years ago. Comparative genomic, phylogenetic and *in silico* structural analyses of animal Class-B 7TMICs reveal high sequence, structure and copy number conservation, suggesting that they are under substantial evolutionary constraint. Single cell/bulk RNA sequencing data and *in vivo* expression analyses suggest that Class-B 7TMICs are—in contrast to Ors/Gr—expressed in a wide variety of tissues. Loss of the *D. melanogaster* homolog does not lead to overt developmental or anatomical phenotypes, but has physiological consequences, including reduced desiccation tolerance, decreased ability to recover from thermal shocks, and lower reproductive output. These ongoing studies raise the possibility that extant 7TMICs are descended from a broadly-expressed, critical ancestral protein, and that the neurosensory role of Ors/Gr is a comparatively recent evolutionary specialization.

Receptor function

Metabotropic signaling broadens odour tuning in Ionotropic receptor neurons

Authors: Suguru Takagi¹; Liliane Abuin¹; Jérôme Mermet¹; Giovanna Lucarelli¹; Daehan Lee²; Richard Benton¹

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A striking dichotomy in olfactory systems exists between mammals, which use G-protein coupled receptors (GPCRs) for odour detection, and insects, which rely on odour-gated ion channels, including the Ionotropic receptor (Ir) repertoire, which is distantly related to synaptic ionotropic glutamate receptor (iGluR) family. Here we report the first evidence for a GPCR signaling pathway in shaping the odour-tuning profile of an insect OSN. Characterisation of the volatile carboxylic acid-sensing properties of *Drosophila melanogaster* Ir64a neurons revealed two subpopulations: one type (DC4) is broadly-tuned to acids of diverse chain length, while the other (DP1m) is narrowly tuned to short-chain acids. Comparative single-cell transcriptomic analysis of DC4 and DP1m populations revealed a G γ subunit, G γ 30A, as being more highly expressed in DC4. We show that G γ 30A is required to broaden the acid-tuning profile of DC4 neurons, acting together with the non-differentially expressed G α s and G β 13F subunits and the adenylyl cyclase, Ac13E. Screening of candidate GPCRs functioning upstream of this metabotropic signaling pathway identified the Latrophilin-family adhesion GPCR, Cirl, whose vertebrate homologues are best-characterised for roles in synapse assembly. Cirl is broadly transcribed in antennal neurons (including both DC4 and DP1m) but, remarkably, only robustly detected at the protein level in Ir64a DC4 neuron ciliated dendrites, a localisation pattern that requires G γ 30A. Importantly, loss of Cirl is also required for the broad tuning of DC4 neurons towards carboxylic acids, while Cirl and Ir64a are not co-dependent for cilia localisation. We further show that in *D. sechellia*, a close relative of *D. melanogaster* with distinct acid preferences, DC4 neurons have reduced breadth of tuning. This interspecific difference cannot be explained by changes in Ir64a sequence nor decreased expression, but is associated with lower expression in *D. sechellia* of two components of this G protein signaling pathway (Cirl and Ac13E). Together, our work reveals the engagement of a metabotropic pathway with ionotropic signal transduction, revealing an unprecedented mechanism defining olfactory sensory neuron tuning properties. More generally, our work underline further molecular parallels between signalling mechanisms in sensory cilia and central synapses.

Receptor function**Chemosensory evolution at the origin of inquilinism in the bee louse fly (*Braula coeca*)****Authors:** Alizée Delarue¹; Amir Yassin²; Héloïse Bastide²; Jean-Christophe Sandoz²; Julie Carcaud¹¹ *Université Paris-Saclay, UMR Évolution, Génomes, Comportement et Écologie*² *Université Paris-Saclay, CNRS, UMR Évolution, Génomes, Comportement et Écologie*

Living beings are not isolated and interact with each other. These interactions are often mediated by chemical odorants but how the evolution of the olfactory system can determine intra- and inter-specific interactions remains unclear. A peculiar example is the queen mandibular pheromone (QMP) in *Apis mellifera*, which inhibits the development of the ovaries in the conspecific workers (hence leading to social cohesion) and also in other insect species (hence protecting the colony). Of the different parasites of *Apis*, only one, the blind and wingless bee louse (*Braula coeca*), paradoxically spends its entire life cycle as an inquiline in the colony, clinging to the thorax or head of bees, with a clear preference for the queen at the end of the summer. We have sequenced its genome and confirmed that it is a basal branch of the Drosophilidae family, closely related to *Drosophila melanogaster*. To understand how *Braula coeca* has found an evolutionary means of circumventing the anti-ovarian effect of QMP, we investigated the evolution of multiple chemosensory gene families. We found the bee louse has lost almost half of the olfactory receptor drosophilid repertoire, including some that respond to QMPs in *D. melanogaster*. By combining multiple functional approaches, including behavioral assays in the field, electroantennography, heterologous gene expression of *Braula* receptors in *D. melanogaster* empty neurons, and CRISPR-Cas9 genome editing to induce *B. coeca* mutations in *D. melanogaster*, we are currently testing the hypothesis that the rapid evolution of *Braula coeca*'s ORs has protected it from the anti-ovarian effects of QMP, enabling it to reproduce in an essentially sterilizing environment. Primary results are supporting this hypothesis, hence providing significant insights on how changes in chemosensory genes promote the evolution of inter-specific interactions.

Receptor function**Alternative strategies based on transgenic *Drosophila melanogaster* for the functional characterization of insect ionotropic receptors****Authors:** Alberto Maria Cattaneo¹; Cristina M. Crava²; William B. Walker III³¹ *C3A - CENTER AGRICULTURE FOOD ENVIRONMENT, UNIVERSITY OF TRENTO*² *University Institute of Biotechnology and Biomedicine, University of Valencia*³ *USDA-ARS Temperate Tree Fruit and Vegetable Research Unit*

Background Insect Ionotropic Receptors (IRs) are a relatively uncharted territory. Some studies have documented IR activation by recording neuronal activity *in situ*, others by their heterologous expression in *Xenopus* oocytes or misexpressing IRs from *Drosophila melanogaster* or from the related *D. sechellia* into the *D. melanogaster* “ionotropic receptor decoder” neuron, which lacks the endogenous tuning receptor subunit but expresses IR-coreceptors.

Results In this study, we first made use of *Drosophila* olfactory sensory neurons (OSNs) different from the “ionotropic receptor decoder”, demonstrating that by replacing or introducing IRs alongside the native *D. melanogaster* ones, functional heteromeric complexes can be formed. IR41a1 from the lepidopteran *Cydia pomonella* exhibits binding to polyamines and the IR75d from the dipteran *Drosophila suzukii* binds hexanoic acid. Secondly, expressing *D. suzukii*'s putative acid sensor IR64a into the “ionotropic receptor decoder” of *D. melanogaster* inhibits the response to the main activators of neighboring neurons from the same sensillum, despite that IR64a does not respond to acids. *In situ* hybridization on the antennae of *D. suzukii* unveils wide expression of IR64a in neurons proximal to the sacculus. Structural modeling analysis does not explain its absence of binding to acids; conversely, this approach identifies key amino acids features explaining the binding of hexanoic acid

by IR75d. Finally, we have also explored alternative methods to heterologously express IRs based on Human Embryonic Kidney cells (HEK293). Despite observing correct expression of IRs in transfected cells through immunohistochemistry experiments, this approach did not achieve successful deorphanization of these receptors.

Conclusions Our findings highlight the potential use of *Drosophila* OSNs as a valuable tool for functional characterization of IRs from different insect species: for the first time, we have provided evidence of IR-functionalities within alternative OSNs from the *Drosophila*'s "ionotropic receptor decoder" neuron to functionally characterize and deorphanize IRs from lineages that are evolutionarily distant from the *D. melanogaster* subgroup, contributing to the understanding of chemosensory modalities in *D. sukukii* and *C. pomonella*, two globally significant agricultural pests. Additionally, the unsuccessful deorphanization in HEK cells highlights the complex requirements for IR functionality, supporting the use of *Drosophila* OSNs as a more suitable expression system.

Perspectives Results from this investigation represent the first step towards a more ambitious approach: in the frame of the ongoing project at the C3A-center of the University of Trento, we claim to isolate neurons from transgenic *D. melanogaster* to transit *in vitro* the functional investigation of the heterologous IRs from several pests of agriculture and forestry, and from other invasive or commercial arthropods.

Insect Vector

Non-canonical olfaction in mosquitoes

Authors: Meg Younger¹; Wesley Alford^{None}; Wei-Chung Allen Lee^{None}; Mohd Freezely^{None}

¹ Boston University

Mosquitoes rely heavily on human-derived chemosensory cues as they search for a blood meal. Understanding how mosquitoes detect and encode human odor would provide a major inroad to prevent mosquito biting behavior and the transmission of diseases that claim more than half a million lives each year. The study of mosquito olfaction also provides an opportunity to address fundamental questions about chemosensory neuroscience in an organism whose behavior is driven strongly by their sense of smell. We discovered that the olfactory system of *Aedes aegypti* mosquitoes has a radically different organization from the canonical "one-receptor-to-one-neuron" organization identified in model species, with widespread chemosensory receptor co-expression within many individual olfactory sensory neurons. We also identify differences in the synaptic connectivity of sensory circuits that underly host detection, as compared to *Drosophila melanogaster*. These dramatic differences in sensory organization have wide-ranging implications for olfactory physiology in general and specifically the detection and integration of human odor cues that support robust human host-seeking.

Insect Vector

Towards exploiting skin bacteria to protect animals against insect vectors

Authors: Alec Hochstrasser¹; Mitchel Bourne¹; Dani Lucas-Barbosa¹; Alexander Mathis¹; Paula Brok¹; Niels Verhulst¹

¹ National Centre for Vector Entomology, Institute of Parasitology, One Health Institute, University of Zurich

Mosquitoes (Culicidae) and biting midges (Ceratopogonidae, genus Culicoides) use carbon dioxide and body odours to find their blood meal host. Skin bacteria play an important role in the production of these body odours, and the human skin microbiota composition correlates with differences in attractiveness to mosquitoes. This opens up the possibility of protecting animals from biting insects by supplementing their skin microbiome with probiotics. We investigated this probiotic approach in an *in vitro* sheep model. Sheep-skin bacteria species were cultured, and experiments using a Y-tube olfactometer and membrane feeding revealed bacterial isolates that repel and inhibit feeding

by biting midges by up to 83%. In a further in vivo study, a repellent bacterial strain was applied directly on sheep skin to test whether this confers protection against biting midges. Just like humans, individual sheep differed in their attractiveness to biting insects. The skin probiotics reduced biting, and the effect lasted for at least three days. Adding the skin probiotic did not alter the rest of the sheep's skin microbiome and did not lead to adverse skin reactions. Skin probiotics could be a novel solution, with longer-lasting protection compared to the topical application of repellents that evaporate within hours.

Insect Vector

Unravelling the sugar-feeding behaviour of *Culicoides* biting midges

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¹ National Centre for Vector Entomology, Institute of Parasitology, One Health Institute, University of Zurich, Switzerland

² Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Sweden

Biting midges of the genus *Culicoides* are vectors of veterinary relevance, as they can transmit a range of pathogens, including bluetongue virus and epizootic hemorrhagic disease virus. Novel control options may include push-pull systems to alleviate the impact of insect vectors on their hosts. While some spatial repellents have proven to be valid “push” candidates, we still lack an effective and selective attractant. Most field-collected biting midges are positive for sugars, an essential energy source for both female and male individuals. Biting midges were collected from field sites around Zürich, Switzerland, and their plant sugar source was analyzed by amplification and sequencing of residual plant DNA from the *Culicoides*' crop. Over 100 clear DNA sequences were retrieved, revealing that *Solanum tuberosum* (potato), and *Glycine max* (soya bean) are recurring plant species in the diet of these insects. Biting midges were able to acquire sugars from these plants in a laboratory single-choice setup. Their survival after three days was also higher in the presence of these species, when compared to other plants. By GC-EAD we will unravel which volatiles might be responsible for the attraction of biting midges to these sugar sources. The identification of attractive compounds can lead to the development of effective baited traps or toxic sugar baits.

Insect Vector

Mechanistic insights into LIPS-2 modulated probing behaviour of the tiger mosquito

Authors: Cecilia Tanzi¹; Felix Hol²; Irene Arnoldi²; Marta Villa¹; Paolo Gabrieli¹; Zhong Wan²

¹ University of Milan

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After landing on the skin of a mammalian host, female mosquitoes start probing this tissue for a blood vessel, salivating and moving the mouthparts in a stereotypical way. Intradermal probing must be fast and efficient, being a necessary but hazardous step in mosquitoes' life cycle, as they need to acquire and digest blood to complete egg development.

We have recently characterised the role of Labrum Interacting Protein of the Saliva (LIPS)-2 in modulating intradermal probing in the tiger mosquito *Aedes albopictus*. LIPS-2 is expressed in female mosquito salivary glands and secreted in the host skin at the bite site. The LIPS-2 gene is restricted to the Culicinae subfamily, including *Aedes* and *Culex* mosquitoes, while is absent in the Anophelinae subfamily. We observed that knocking down the expression of LIPS-2 protein in *Ae. albopictus*

female mosquitoes increased their probing time while feeding on a human volunteer. Here, we combine genetic engineering, electron microscopy imaging, and behavioural assays to gain mechanistic insight into this phenotype.

LIPS-2 binds to the tip of the labrum, the elongated and sharp-pointed stylet forming the food channel within the mosquito mouthparts. LIPS-2 binding leads to a modification of the height of labral ridges, two cuticular structures located at the tip of the labrum. These structures can be observed in *Ae. albopictus*, *Aedes aegypti*, and *Culex pipiens* mosquitoes, but are absent in *Anopheles stephensi*, as assessed by scanning electron microscopy. Labral ridges were previously described to each host one pair of sensory dendrites called labral ridges receptors (lrr). Despite being suggested to have a proprioceptive role, the function of these structures in mosquito sensation during feeding remains to be understood.

To gain mechanistic understanding in the functioning of LIPS-2 in modulating probing dynamics, we characterized a LIPS-2 knockout line using the BiteOscope, a platform allowing the quantification of landing, probing, and engorging of mosquitoes on modifiable synthetic substrates. Additionally, electropenetrography was implemented to observe, record, and quantify probing and engorging behaviours in the LIPS-2 knockout line. Together, these experiments represent a base for a deeper understanding of the function of LIPS-2 in the modulation of intradermal probing in *Aedes* mosquitoes.

Insect Vector

Breaking the unbreakable: Dissecting DEET and picaridin's behavioral and neuronal basis in mosquitoes

Authors: Jacopo Razzauti¹; Leslie B. Vosshall¹; Petr Skovorodnikov¹; Tianyi Wei¹

¹ *The Rockefeller University*

Female *Aedes aegypti* mosquitoes employ remarkably robust, seemingly “unbreakable” host-seeking behavior to detect and bite humans, making them highly effective vectors for dangerous arboviruses including dengue, Zika, and chikungunya. While insect repellents containing DEET and picaridin effectively disrupt this persistent attraction and prevent bites, their precise mechanisms of action remain unclear. DEET has been demonstrated to possess both volatile and contact modes of repellency, but despite various hypotheses explaining how repellents function, the inability to disentangle their multimodal effects has hindered our understanding of the underlying sensory mechanisms.

To address this challenge, we developed the mosquito HOSTel—a novel behavioral chamber enabling precise spatiotemporal control of stimulus delivery with independent manipulation of contact and non-contact delivery modes. Coupling this system with a custom-made, open source, deep learning-based tracking platform capable of monitoring large numbers of individual mosquitoes without identity loss, we demonstrate that picaridin functions primarily as a contact-based repellent with orco-independent action and spatially circumscribed effects.

To investigate the sensory basis of these behavioral differences, we are employing in vivo calcium imaging of mosquito leg sensory neurons to examine how picaridin and DEET may engage different chemosensory pathways to disrupt host-seeking behavior. This integrated approach combining behavioral quantification with physiological investigation aims to elucidate how these leading repellents break one of nature's most resilient and epidemiologically significant behaviors.

Insect Vector

Decoding responses in the olfactory system of the yellow fever mosquito

Authors: Benjamin Fabian¹; Sukritha Nalikkaramal²; Silke Sachse¹; Rickard Ignell³; Bill S. Hansson⁴

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Aedes aegypti, the primary vector of several arboviruses including those that cause dengue, Zika, and yellow fever, relies heavily on olfactory cues to locate human hosts for blood feeding. The mosquito's olfactory system is characterized by significant redundancy, with individual olfactory sensory neurons co-expressing multiple receptor types. This redundancy poses both a challenge and an opportunity for understanding the neural basis of host-seeking behavior. Recent advances in genetic tools have enabled the development of *Aedes aegypti* strains expressing the calcium indicator GCaMP in defined olfactory neuron populations. Leveraging these tools, we perform *in vivo* volumetric calcium imaging using advanced multiphoton microscopy to map the activation patterns within the antennal lobe in response to volatile odorants known to elicit behavioral responses. These experiments will elucidate the specific glomeruli involved in detecting distinct olfactory cues, uncover patterns of sensory encoding, and offer insights into the extent of functional redundancy within the mosquito's olfactory circuitry. In subsequent experiments, we will explore potential synergistic effects between carbon dioxide and individual human odorants or odor blends. Understanding how combinations of host-derived cues are integrated at the neural level will shed light on the mechanisms that underlie host attraction and may inform novel strategies for vector control.

Insect Vector

How experience can drive oviposition preference in insects

Authors: Eduardo Briceño-Aguilar¹; Julio Otárola-Jiménez¹; Markus Knaden¹

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Most insects do not provide any direct care for their offspring. Therefore, it is crucial that female insects carefully judge oviposition sites and lay their eggs at places that provide good conditions for their offspring's survival. To do so, ovipositing insects often evaluate the suitability of an oviposition site based on innate preferences. However, we recently could show that in addition oviposition experience can drive the female insects' decision. Here I will show how such experience can influence oviposition behavior in flies and moths and can finally result in "oviposition constancy", i.e. a behaviour similar to "flower constancy" observed in bees.

Poster abstracts

Poster Session 1 / odd - Poster number: 1

VOC induced interactions of *Gasterophilus* bot flies with Welsh ponies in France

Authors: Marielle Zayana¹; Fabrice Reigner²; Elfie Perdereau³; Fotini Koutroumpa⁴

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Gasterophilus spp. and gastrointestinal nematodes are among the main endoparasites affecting equines worldwide. Due to their recurrent presence in the host and the challenges associated with their diagnosis, treatments are typically administered once or twice a year using broad-spectrum antiparasitic drugs to target both parasites simultaneously. However, the growing issue of antiparasitic resistance, particularly among nematodes, has become a major concern. In the absence of data on the chemical interactions between equines and their endoparasites, we investigate whether these parasites can alter the equine volatilome. Specifically, we aim to determine whether volatile organic compounds (VOCs) can serve as reliable diagnostic markers to distinguish between infested and non-infested horses. This approach could help guide more targeted treatments and potentially delay the development of resistance.

Poster Session 2 / even - Poster number: 2

Untangling space and taste within the sensory world of *Drosophila*

Authors: Alexi Mery¹; Darren Williams²; Jason Somers¹; John Tuthill³; Leila Elabbady³; Lucia Prieto-Godino¹

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Organisms rely on multi-sensory integration to perform appropriate behaviours. A powerful example of this is the insect leg gustatory system, where knowledge about the quality and the location of a tastant is important. However, we do not currently know how these distinct information streams are integrated. To investigate the possible existence of a dual chemosensory and topographic map, we study the *Drosophila melanogaster* leg –a unique sensory organ on which chemosensory and mechanosensory sensilla overlap. We propose that *Drosophila* leg chemosensory neurons are able to convey both the location and the quality of a tastant. First, we are performing thermogenetic experiments to determine whether such maps exist, followed by using clonal labelling tools and connectomics to understand how these maps might be organised. From this, we see segregation of chemosensory neuron projections from different leg compartments, along with morphologies that correlate with the receptor identity of a taste neuron. Put together, our work is aiming to uncover how the anatomy and receptor identity of sensory neurons are used to convey different types of information to downstream circuits.

Poster Session 1 / odd - Poster number: 3

Uncovering the chemosensory repertoire of *Ips typographus* larvae: gene annotation and expression profiling

Authors: Anna Magnusson¹; Martin N. Andersson¹

¹ Lund University

The Eurasian spruce bark beetle (*Ips typographus*) is both a keystone species and a major pest in spruce forests across Europe and Asia, contributing to large-scale damage during epidemic outbreaks. Previous studies on insect chemosensation, including bark beetles like *I. typographus*, have been biased towards the adult life stage, while the larval sensory biology is largely unexplored, particularly in Coleopterans. This is a knowledge gap worth considering, as larvae, together with symbiotic fungi, are directly responsible for the phloem degradation that causes spruce tree mortality.

In this study, we focus on the annotation and gene expression profiling of chemosensory genes in two instars of *I. typographus* larvae. Using available genome and adult antennal transcriptome assemblies, we have manually identified candidate gene models for their genomic position and exon-intron boundaries. Previous chemosensory gene annotation in *I. typographus* have been partial. Here, we provide a more complete set of candidate genes from key chemosensory gene families, including odorant receptors (ORs), gustatory receptors (GRs), ionotropic receptors (IRs), odorant binding proteins (OBPs), chemosensory proteins (CSPs) and sensory neuron membrane proteins (SNMPs).

Gene expression level analysis and identification of larval-specific chemoreceptors are currently underway using data from high-throughput RNA sequencing of heads from first- and third-instar larvae. These results will guide the functional characterization of larval-specific chemoreceptors and behavioral assays and will also be compared to adult expression profiles to reveal potential developmental shifts in chemosensory function across the *I. typographus* life cycle.

Poster Session 2 / even - Poster number: 4

How subpopulations of MBONs integrate olfactory and visual information across sub-regions of the calyx

Authors: Athil Althaf Aliyam Veetil Zynudheen¹; Wolfgang Rössler²; Martin Strube-Bloss³

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Different types of classical and operant conditioning experiments have shown that honeybees, as central place foragers, have a vast repertoire of stimuli that they can associate with the rewarding nectar and pollen of a flower. These associations can be unimodal, like the odour or visual component of a blossom, but they can also combine both modalities, and bees learn that only the olfactory-visual compound is rewarded and not its single elements.

By electrophysiologically recording mushroom body (MB) output neurons (MBONs), we focus on the sensory integration of both modalities. At this processing level, some subpopulations of MBONs respond to both modalities, whereas other subpopulations respond to either light or odour (Strube-Bloss and Rössler, Roy.Soc.OpSci 2018). Moreover, some MBONs send retrograde centrifugal feedback innervating the antennal lobe (AL). Thus, they may modulate olfactory processing within the AL. Before we address how cross-modal learning experiments will modulate single MBONs, we aim to understand how the general unimodal and multimodal MBONs code at this processing level. When we presented the bees with different lights, odours and the corresponding compound stimuli, we observed that superficial unimodal MBONs, which responded to only one modality (odour or light), responded differently when stimulated with the compound, suggesting a modulatory interaction.

Morphological studies have shown that olfactory projection neurons innervate the MB's lip and basal ring, while visual projection neurons innervate the calyx's collar and basal ring region.

We implemented these plausible connectivities into a rate-based model. We assumed that different populations of Kenyon Cells (KCs) relay the information of these regions in parallel, before MBONs integrate across different KC subpopulations. Unimodal MBONs have to be connected with the KC population of the individual single modality and to the basal ring. The latter multimodal pathway then dictates the modulatory effect. By combining modelling and previously known connectivity within the MB, we could predict the observed phenomena and infer plausible connectivity of MBONs with KCs.

Poster Session 1 / odd - Poster number: 5

Neuromodulation of odor-guided feeding motivation and odor preference in the hungry fruit fly *Drosophila melanogaster*

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Olfaction is crucial for appetitive search in hungry insects, as odor plumes carry information about food quality and location. Hunger enhances sensitivity and preferences to food-related odors, presumably to maximize the chance of meeting nutritional demands. However, how hunger promotes odor-guided appetitive search into environments with limited accessibility is unexplored. To address this question, we developed a behavioral paradigm using a custom Y-maze with a narrow passage connecting the entrance to the choice point, allowing simultaneous analysis of odor preference and odor-guided exploration through the narrow passage. Using this setup, we observed that hungry fruit flies *Drosophila melanogaster* are more likely guided to enter the narrow passage in the Y-maze, while most fed flies tend to remain not exploring. Compared to the fed group, the entry ratio of hungry flies to the choice point significantly increased with longer starvation hours and higher concentrations of an appetitive odor, suggesting feeding motivation. In a preliminary genetic screen to uncover the underlying neuromodulation mechanisms, we identified several neuropeptide mutants that showed higher entry ratios even when fed, suggesting a critical role of these neuropeptides in setting hunger state or odor sensitivity. This paradigm offers a new approach to uncover the mechanisms of hunger-driven odor preference and voluntary entry as a proxy for motivational regulation.

Poster Session 2 / even - Poster number: 6

Expression of olfactory proteins in tarsal neurons of the desert locust *Schistocerca gregaria*

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The desert locust *Schistocerca gregaria* is capable of forming destructive swarms of millions of individuals, causing enormous agricultural and economic damages. The behaviours of this species, such as swarm formation and food search, are largely driven by chemosensory cues from the environment. In insects, including *S. gregaria*, the role of odorant receptors (ORs) expressed on the antennae in the detection of relevant odorants and pheromones has been extensively demonstrated. However, transcriptome and reverse transcription-PCR (RT-PCR) analyses revealed ORs also in other parts of the insect body, including tarsi, wings, and the ovipositor. The functional relevance of OR-expressing cells apart from the antenna is still largely elusive. As a first step to elucidate the functional implication(s) of "extra antennal" OR-positive cells in *S. gregaria*, we aimed at identifying olfactory proteins in other tissues of the desert locust. RT-PCR analyses revealed expression of the odorant receptor co-receptor (ORCO) in various locust body parts implicated in chemosensory functions in other insects, including the tarsi. Through fluorescence immunohistochemistry on tarsal sections with

antibodies against ORCO and a neuronal marker, ORCO was found to be localized in neurons associated with distinct sensilla types, suggesting a chemosensory function of these cells and sensilla. Moreover, by RNA sequencing and RT-PCR we identified a number of OR-types abundant in tarsal tissue. In ongoing work we try to localize cells in the tarsi expressing these OR-types. To target the potential chemosensory function of identified neurons in the tarsi, we intend to employ OR/ORCO knockdown strategies (RNAi) in combination with electrophysiological approaches.

Poster Session 1 / odd - Poster number: 7

Cytochrome p450s in the *Drosophila* olfactory system

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Insects utilize their antennal olfactory system to detect numerous environmental odors to aid in behaviors such as identifying food sources, host seeking, and mating. While Olfactory Receptor Neurons (ORNs) have been extensively studied, much less is known about the tormogen, trichogen, and thecogen support cells that are co-housed in olfactory sensilla. The *Drosophila melanogaster* antennal transcriptome contains many putative Odorant Degrading Enzymes (ODEs), and support cells are thought to use these to metabolize odors. One abundant class of antennal ODEs is the Cytochrome P450 (Cyp) gene superfamily. However, their cellular localization and potential contribution to odorant responses is poorly understood. Here, we investigated the localization of thirteen antennal Cyps, including those most highly expressed and those with antennal-enriched expression compared to other tissues. Using newly generated transgenic LexA reporter lines, we found that most Cyps are broadly expressed across the antenna, whereas a few have more restricted expression. While expression of a few Cyps may be limited to the antenna, many are additionally expressed in the maxillary palp or the Johnston's organ. Further localization of one narrowly-expressed Cyp, Cyp313a4, revealed that it is exclusively expressed in two subtypes of coeloconic sensilla, suggesting it may contribute specifically to their olfactory function. The expression of Cyps in different antennal cell types was investigated using markers for neurons (anti-elav), thecogen cells (anti-pros), trichogen cells (atk-Gal4), tormogen cells (ASE5-Gal4), and epithelial cells (Obp19d-Gal4). Among support cells, Cyps were predominantly expressed by trichogen cells, with some expression in tormogen cells and rarely thecogen cells. Unusually, Cyp313a1 is mostly expressed by a subset of olfactory neurons; no other Cyps have neuronal expression. In addition to support cell expression, many Cyps are also expressed by epithelial cells. Together, this work presents the first map of Cyp expression in the *Drosophila* antenna and can serve as a roadmap for future studies on their functional roles.

Poster Session 2 / even - Poster number: 8

The chemical and sensory bases of social immunity in the clonal raider ant

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Social insects live in densely populated colonies, which increases the risk of disease outbreaks. As a result, they have developed collective strategies for disease defense known as 'social immunity.' For instance, social insects groom infectious fungal spores from each other's body surfaces to prevent infection. Social immunity depends on the ability to identify infected conspecifics, but the chemical and sensory bases of this detection remain poorly understood.

We aim to explore the mechanistic bases of social immunity using the queenless clonal raider ant *Ooceraea biroii*, a blind species that likely detects sick nestmates by olfactory cues. However, other

modalities (e.g., gustation) might also play a role in detecting sickness-related cues. Our objectives are to 1) identify candidate olfactory “disease-related cues” by comprehensively screening the chemical profiles of infected ants, 2) characterize the behavioral responses of workers towards nestmates infected with different pathogens and using this response to functionally test candidate chemicals, and 3) investigate how “disease-related cues” are encoded in the brain through in vivo calcium imaging of the antennal lobe. Additionally, we will examine neuronal responses to pathogen odors and compounds that are known to trigger social immunity behavior (e.g., ergosterol). Preliminary behavioral data indicate a response of non-infected nestmates towards bacteria-infected individuals at specific time points following bacterial infection. The chemical analysis of bacteria-infected ants revealed several candidate compounds that could be involved in eliciting the observed behavior towards infected individuals.

By combining chemical, neuronal, and behavioral analysis, we anticipate shedding light on the mechanistic bases of social immunity in insect societies.

Poster Session 1 / odd - Poster number: 9

Markers of olfactory sensilla support cells in adult *Drosophila*

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In the *Drosophila* antenna, olfactory sensilla are the fundamental functional unit of odor detection and processing. These sensilla house not only the well-studied Olfactory Receptor Neurons (ORNs), but also three distinct support cells: the thecogen, trichogen, and tormogen cells. Our understanding of the role of support cells in olfactory signaling has been limited by the lack of markers to distinguish these cells in adult flies. Genetic markers for these cell types have been identified for developmental studies, but their specificity and expression in the adult antenna remains poorly defined. Here, we systematically identified and validated tools to label the three support cell types as well as the epithelial cells found between sensilla. First, we confirmed previous suggestions that *ASE5-GAL4* specifically and broadly labels tormogen cells, which express Su(H). In contrast, expression of *nompA-GAL4*, previously used as a thecogen cell marker, is strongly biased toward coeloconic sensilla and does not capture all thecogen cells. As an alternative, we identified prospero as a candidate thecogen marker using the Fly Cell Atlas. We found that an anti-prospero antibody widely labels thecogen cells, which are ASE5-negative. Because an adult trichogen cell marker has not been previously reported, we generated knock-in *GAL4* lines for two candidate genes identified through a literature search. One line, *atk-GAL4*, broadly drives expression in cells that are distinct from those expressing ASE5 or prospero (which are also distinct from each other), supporting their identity as trichogen cells. Finally, we examined an *Obp19d-GAL4* line based on prior reports that Obp19d is predominantly expressed in epithelial cells. Like the support cell markers, *Obp19d-GAL4* widely labels non-neuronal cells. The Obp19d-positive cells are also distinct from support cells, confirming their epithelial identity. Together, our findings provide a validated framework for distinguishing antennal support cell classes in the adult fly, a crucial step for understanding their individual roles in olfactory sensilla.

Poster Session 2 / even - Poster number: 10

A deep learning model for VOC-driven mating analysis in BSF

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Volatile organic compounds (VOCs) are increasingly recognized as key modulators of insect behavior, yet their impact on courtship dynamics remains understudied. Here, we present a deep learning-based behavioral tracking system tailored to detect and quantify mating events in *Hermetia illucens* (Black Soldier Fly) under controlled experimental conditions. This tool enables high-throughput, fine-scale analysis of behavioral responses to environmental variables. Select VOCs, previously shown to elicit physiological responses in BSF, were chosen for behavioral testing to probe their influence on mating activity. The system is currently being applied to assess the effects of VOC exposure, cage density, and sex ratio on mating rates. By integrating computer vision with behavioral ecology, this approach opens new avenues for investigating the role of semiochemicals in insect reproduction and lays the groundwork for chemically informed strategies in insect mass-rearing and behavioural research.

Poster Session 1 / odd - Poster number: 11

Odor detection in complex mixtures following appetitive and aversive conditioning

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Odorants are detected by olfactory receptor neurons (ORNs) that project to the antennal lobe (AL), the first olfactory neuropil in the insect brain. In the AL, ORNs make synaptic contacts with: i) projection neurons (PNs), which in turn send olfactory information to other brain areas; and ii) local interneurons (LNs) that form a dense network of lateral inhibitory and excitatory interactions within the AL. Functional and computational studies indicate that this local network transforms sensory input, presumably to enhance perception of meaningful odors.

Here, we investigate the role of GABAergic local neurons in both learning-dependent plasticity in the AL and the ability of flies to perceive the presence of learned odorants in a mixture. For that aim, we performed aversive olfactory conditioning using a single odorant as a conditioned stimulus. Next we tested olfactory preference in a T-maze by exposing the flies to the conditioned odorant, or to the conditioned odor embedded in a mixture of different proportions with a novel odorant (distractor). We determined the proportion threshold that flies need to detect the learned odorant immersed in the mixture. Next, we demonstrated that blocking the activity of different groups of LNs in the AL impairs the ability to detect the learned odorant embedded in mixtures. We then asked whether aversive conditioning affects the representation of odorant mixtures in the AL. We recorded odorant evoked responses in PNs using calcium imaging, while concomitantly training animals with the same protocol used in the T-maze experiments. We were able to study odorant representation before and after training, and found that the representation of a binary mixture in the AL changes after the learning.

Finally, we are investigating whether this type of experience-dependent plasticity is conserved in an appetitive conditioning. For that, we paired a single odorant with sucrose and test attraction preference to a binary mixture that contains this odor. We then evaluated whether the detection of target odors in mixtures requires the activity of local neurons.

Poster Session 2 / even - Poster number: 12

Sucrose sensitivity and dietary signal integration shape feeding decisions in whiteflies

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Sweet taste sensation plays a critical role in feeding-site selection, dietary evaluation, and appetite regulation in insects. This capability is particularly vital for phloem-feeding insects, as their feeding site lies deep within the leaf and contains extremely high concentrations of sugars, primarily sucrose, that can pose osmoregulatory challenges. The molecular basis of sweet taste perception relies on the activity of gustatory receptors. In previous work, we demonstrated that *Bemisia tabaci* Gustatory Receptor 1 (BtabGR1) is highly sensitive to sucrose and contributes to feeding-site selection, as shown using artificial diet assays. Building on these findings, we now investigate how additional dietary compounds influence the feeding behavior of *B. tabaci*. Our recent behavioral and pharmacological studies suggest that interactions among multiple dietary cues generate integrated sensory signals, allowing whiteflies to fine-tune their feeding strategies.

Poster Session 1 / odd - Poster number: 13

Attraction to date extract in *Aedes* mosquitoes reveals species-specific foraging strategies

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Aedes aegypti and *Aedes albopictus* are major mosquito species that transmit arboviruses such as dengue, Zika, chikungunya, and yellow fever. While their host-seeking behavior is well studied, less is known about their use of plant-derived sugar sources, which are essential for survival and daily activity. Date extract is known to attract mosquitoes, but the specific volatile compounds responsible for this response remain unclear. Volatile compounds were identified via GC-MS analysis of date extracts. The goal is to isolate a minimal set of odorants that consistently attract both species and deepen our understanding of mosquito olfactory ecology.

This study explores the olfactory-guided behavior of *Aedes* mosquitoes in response to different odorants present in date extract. We used behavioral assays featuring custom 3D-printed traps of various shapes and sizes, along with a fine-tuned object detection model, to quantify attraction. Initial results suggest species-specific preferences for trap types, possibly reflecting different foraging strategies. These findings provide a basis for broader investigations into mosquito foraging strategies and sensory decision-making.

Poster Session 2 / even - Poster number: 14

Integration of learning in an innate pheromone circuit

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Odor-driven innate behaviors dictate an animal's survival and reproduction. *Drosophila* females exhibit genetically driven, stereotyped behaviors in response to the male-produced pheromone 11-*cis*-Vaccenyl Acetate (cVA). This pheromone innately guides mating, aggregation, and other crucial social behaviors and is conserved across several *Drosophila* species. Our initial behavior experiments suggest that classical olfactory conditioning can overwrite the valence of highly ecologically relevant chemical cues such as cVA. We propose that recurrent connections between olfactory neuropils, which harbor innate (Lateral horn) and learned (Mushroom body) memory processing, mediate these modulations in behavior. By the help of transgenic binary expression systems, split-gal4 lines, two-photon calcium imaging techniques, and the connectomic datasets, we trace this narrowly-tuned olfactory circuit that detects cVA and investigate possible connections via Projection neurons (PNs), Mushroom body output neurons (MBONs), and Lateral horn neurons (LHNs) to establish the complex circuits that integrate learning into hardwired representations governing the insect's response to a stimulus. All our experiments are conducted in female *Drosophila melanogaster*.

Poster Session 1 / odd - Poster number: 15

Discovery of a novel Mosquito repellent chemical from perfumery palette

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Mosquito-borne diseases continue to pose significant health risks globally, necessitating the development of effective and sustainable mosquito repellents. The perfumery industry has registered a wide range of diverse aromatic ingredients, that offers a potential source for new anti-mosquito compound discovery. This study explores the mosquito repellent properties of various perfumery ingredients and reports the discovery of a novel repellent compound.

A selection of aromatic compounds commonly used in perfumery was screened for mosquito repellent activity against *Aedes aegypti* and *Anopheles gambiae* mosquitoes. Laboratory-based repellency tests were conducted on both mosquito species. The tests measured the duration and effectiveness of repellency under different controlled conditions. Comparative analysis was performed against commercially available synthetic repellents, DEET and IR3535.

Several perfumery compounds exhibited significant mosquito repellent properties. Among them, a previously unidentified chemical, designated as Lilyflore® demonstrated superior repellency. Comparative studies showed that Lilyflore®'s efficacy was equivalent at isodose to that of DEET and more repellent than IR3535, with additional benefits of pleasant fragrance.

The discovery of Lilyflore® from the perfumery palette opens new avenues for the development of effective and aesthetically pleasing mosquito repellents.

Poster Session 2 / even - Poster number: 16

Evolution of feeding preference in drosophilids

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Food choice is an important driver of speciation and exploration of novel ecological niches. However, we know little about the mechanisms leading to shifts in food preference at the cellular and molecular level. To study this phenomenon, we use the three closely related *Drosophila* species, *D. sechellia*, *D. simulans* and *D. melanogaster*, which dramatically differ in their feeding habits. *D. sechellia*, a host specialist, spends its whole life cycle on a single fruit (*Morinda citrifolia*, noni) - the latter two are generalists living on various substrates. Using several quantitative feeding assays, we can recapitulate the preference for noni in *D. sechellia*. Using neurogenetics, we identify sweet and bitter sensing neurons as the main drivers for this shift in behaviour and compare their physiology between species. We establish a causal link between genetic changes in one single gustatory receptor, peripheral neuron responses in bitter-sensing cells of the labellum and behavioural divergence between species. Through volumetric Calcium imaging in the ventral brain, we detect that, in addition to peripheral physiology, species-specific processing of noni and sugars detection in sensorimotor circuits alters feeding preference. Our data support a model where multiple species-specific modifications lead to altered food consumption across closely related species.