



CHEMICAL COMMUNICATION
IN ECOLOGICAL SYSTEMS



22nd IMPRS Symposium

March 21 – 22, 2023

Guest speakers



Alexander
Haverkamp



Aileen
Berasategui

Please join us for:

- *2 guest talks*
- *23 PhD talks*
- *30 poster presentations*
- *4 awards*

Program

Tuesday, 21st, 2023

07:45	Bus departs for Dornburg (Busbahnhof Jena)
08:45	Welcome (Großer Kaisersaal) <i>Claudia Voelckel</i>
09:00	New arrivals and defended theses in 2022/2023 (Großer Kaisersaal) <i>Sarah O'Connor</i>
09:15	Plenary Lecture 1 (Großer Kaisersaal) "The neuro-ecology of the large cabbage white butterfly: From receptors to the brain" <i>Alexander Haverkamp</i> , Abstract page 9 <div style="text-align: right;"><i>Chair: Markus Knaden</i></div>
	Coffee break (Rittersaal)

1st talk session (Großer Kaisersaal) <div style="text-align: right;"><i>Chair: Kalpana Jain</i></div>	
<i>The architecture and function of smell in insects</i>	
10:30	1 - Response plasticity of <i>Drosophila</i> olfactory sensory neurons <i>Eric Wiesel (HAN)</i> , Abstract page 11
10:45	2 - Exploring the olfactory neuroecology of <i>Drosophila busckii</i> <i>Venkatesh Pal Mahadevan (HAN)</i> , Abstract page 12
11:00	3 - Learnt oviposition preference in the vinegar fly <i>Drosophila melanogaster</i> <i>Julio Otarola Jimenez (HAN)</i> , Abstract page 13
11:15	<i>Stretch your legs break</i>
11:25	4 - Anatomical and cellular organisation of the olfactory system of <i>Locusta migratoria</i> <i>Eleftherios Dimitriou (HAN)</i> , Abstract page 14
11:40	5 - Evolution of sex pheromone receptors in <i>Drosophila</i> flies <i>Melissa Diaz-Morales (HAN)</i> , Abstract page 15
11:55	<i>Lunch break</i> (Rittersaal)

1st poster session (odd numbers)	
<i>Chair: Claudia Voelckel</i>	
13:00	One minute poster talks (1,3,5,7,9,11,13,15,17,19,21,23,25,27,29) (Großer Kaisersaal)
13:20 – 14:50	Poster session (Rittersaal)
14:50	Coffee break (Rittersaal)
14:55 – 15:25	IMPRS faculty meeting (Bibliothek 1)

2nd talk session (Großer Kaisersaal)	
<i>Chair: Akanksha Gandhi</i>	
<i>The making of plant metabolites and their passage through trophic levels</i>	
15:30	6 - Hijacking primary cell component for steroidal specialized metabolism <i>Marianna Boccia (SOC), Abstract page 16</i>
15:45	7 - Reinventing pathways: Convergent evolution of specialized metabolites in flowering plants <i>Matilde Florean (SOC), Abstract page 17</i>
16:00	8 - Uncovering the biosynthesis of gramine in barley and reporting a new UPLC-FLD method for its detection <i>Sara Leite Dias (D'Auria lab), Abstract page 18</i>
16:15	<i>Stretch your legs break</i>
16:25	9 - Exploring biosynthesis and regulation of triterpenoids in <i>Nicotiana attenuata</i> revealed a herbivore-defense function of pentacyclic triterpenes <i>Caiqiong Yang (ITB/SOC), Abstract page 19</i>
16:40	10 - Investigating the costs and benefits of a host plant-dependent chemical defense in a herbivorous insect <i>Johannes Körnig (KAL/Beran lab), Abstract page 20</i>
16:55	11 - Glucosinolates at the next trophic level: Effects of aphid-sequestered compounds on a ladybird predator <i>Tam Mai Duc (GER), Abstract page 21</i>
17:10	<i>End</i>
17:30	Bus departs for Jena

Wednesday, March 22nd, 2023

08:00	Bus departs for Dornburg (Busbahnhof Jena)
09:00	Plenary Lecture 2 (Großer Kaisersaal) “Leaf beetle propagates a plant pathogen in exchange for pupal protection” <i>Dr. Aileen Berasategui, Abstract page 10</i> <i>Chair: Kristina Kshatriya</i>
09:45	Coffee break (Rittersaal)

3rd talk session (Großer Kaisersaal)	
<i>Chair: Emily Puckett</i>	
Endosymbiotic and endoparasitic microbes of insects and plants	
10:15	12 - Gut symbiont transmission in bark beetles <i>Ana Patricia Baños Quintana (KAL), Abstract page 22</i>
10:30	13 - Protecting symbiotic bacteria: A novel role of host-derived cuticular hydrocarbons <i>Bernal Matarrita Carranza (KAL), Abstract page 23</i>
10:45	14 - Inhibition of the shikimate pathway by glyphosate disrupts the stability of symbiosis and impairs host fitness across diverse grain pest beetles <i>Nomthi Khanyile (KAL), Abstract page 24</i>
11:00	Stretch your legs break
11:10	15 - Symbiont interactions and chemical mediators in a defensive symbiosis <i>Alina Nick (KAL), Abstract page 25</i>
11:25	16 - Nematodes dampen division of labour in ants <i>Zimai Li (Ulrich lab), Abstract page 26</i>
11:40	17 - Impact of a novel <i>Trichoderma</i> strain on the host's physiological response to salt stress <i>Akanksha Gandhi (FSU, Oelmüller lab), Abstract page 27</i>
11:55	Lunch break (Rittersaal)

2nd poster session	
<i>Chair: Claudia Voelckel</i>	
13:00	One minute poster talks (2,4,6,8,10,12,14,16,18,20,22,24,26,28,30) (Großer Kaisersaal)
13:20 – 14:50	Poster session (Rittersaal)
14:50	Coffee break (Rittersaal)

4th talk session (Großer Kaisersaal)	
Chair: Zimai Li	
QTL analyses of insect behavior, color and pheromones	
15:20	18 - The evolution of a dual function component in a noctuid moth <i>Elise Fruitet (HEC/Groot lab)</i> , Abstract page 28
15:35	19 - Color morph variation in the meadow grasshopper <i>Pseudochorthippus parallelus</i> is controlled by a few genetic loci <i>Mahendra Varma (FSU, Schielzeth lab)</i> , Abstract page 29
15:50	20 - "Why so (un)predictable?" Correlates of (un)predictability in escape behavior in a grasshopper <i>Gabe Winter (FSU, Schielzeth lab)</i> , Abstract page 30
16:05	<i>Stretch your legs break</i>
Chemical ecology potluck	
16:15	21 - The role of FLC-like genes in <i>Aethionema arabicum</i> <i>Renu Sharma (FSU, Theißen lab)</i> , Abstract page 31
16:30	22 - Selection pressure by specialist and generalist insect herbivores leads to optimal constitutive plant defence - a mathematical model <i>Suman Chakraborty (FSU, Schuster lab)</i> , Abstract page 32
16:45	23 - Desert ants build their own landmarks in a featureless environment <i>Marilia Freire (HAN)</i> , Abstract page 33
17:00	End All jury members submit their scores and rankings (hand to Anja or Claudia)
17:20	Bus departs for Jena

PhD Meeting (MPI for Chemical Ecology)	
18:00	Dinner (Cafeteria)
18:30	Introduction PhD representatives (A1.011 + A1.009)
19:00	Award ceremony <i>Sarah O'Connor</i>

Posters

1. To oviposit or not to oviposit: a host selection dilemma by *Manduca sexta*
Eduardo Briceño Aguilar (HAN), [Abstract](#) page 35
2. Effect of hybridization on the plant metabolome
Olga Zafra Delgado (GER/SOC), [Abstract](#) page 36
3. Adaptive plasticity in specialists and generalists
Somasundar Arumugam (Sachse lab), [Abstract](#) page 37
4. Sex and position in the canopy determine the chemodiversity of the leaves in old-growth black poplar (*Populus nigra*) trees
Sol Yepes Vivas (GER), [Abstract](#) page 38
5. Dissecting odor mixture interactions in the fly brain
Pramit Bandyopadhyay (Sachse lab), [Abstract](#) page 39
6. Chemical signaling in alga-parasite interactions in aquatic ecosystems
Ruchicka O'Niel (FSU, Pohnert lab), [Abstract](#) page 40
7. From land to water and back again: Neuroanatomical adaptations of the olfactory system in Hemiptera insects
Eleftherios Dimitriou (HAN), [Abstract](#) page 41
8. Evolution of fruit scent in Madagascar's figs
Linh Mai Nhat Nguyen (FSU, Nevo lab), [Abstract](#) page 42
9. Evolution of the olfactory system in feeding versus non-feeding moths
Marina Quadrado (HAN), [Abstract](#) page 43
10. The interface of host-symbionts interaction
Dongik Chang (KAL), [Abstract](#) page 44
11. Manipulating the sterol metabolism in tomato for the production of vitamin D3
Marianna Boccia (SOC), [Abstract](#) page 45
12. Creating a genetically tractable symbiosis: Experimental symbiont replacement in grain pest beetles
Ronja Krüsemmer (KAL), [Abstract](#) page 46
13. Development of single-cell mass spectrometry method for the study of the medicinal plant *Catharanthus roseus*
Anh Hai Vu (SOC), [Abstract](#) page 47
14. Endosymbionts contribution to the cuticle synthesis of a pest beetle
Camila Fiori Pereira (KAL), [Abstract](#) page 48
15. Metabolism of spruce phenolic defense compounds by a symbiotic fungus of bark beetles
Baoyu Hu (GER), [Abstract](#) page 49
16. Dynamics and mechanisms aiding symbiont colonization in a darkling beetle
Ramy Ganesan (KAL), [Abstract](#) page 50
17. An evolved gamma-aminobutyric acid aminotransferase drives steroidal glycoalkaloid biosynthesis in *Solanum*
Dagny Grzech (SOC), [Abstract](#) page 51
18. Impact of the microbiome on *Drosophila melanogaster* cold tolerance
Marion Lemoine (KAL), [Abstract](#) page 52
19. The formation of iridoid trail pheromones in the Argentine ant *Linepithema humile*
Maithili Datta (SOC), [Abstract](#) page 53

20. Effect of nematode infections on nestmate-recognition in clonal raider ants
Bhoomika Bhat (Ulrich lab), [Abstract](#) page 54
21. Toxicity of cardenolides from *Asclepias curassavica*
Paola Andrea Rubiano-Buitrago (ROW), [Abstract](#) page 55
22. saxA as public good? - Rethinking the role of pathogens in leaf microbiomes
Kerstin Unger (FSU, Agler lab), [Abstract](#) page 56
23. Identification of plant 17,20- α -desmolases
Glendis Shiko (FSU, Oelmüller lab), [Abstract](#) page 57
24. Targeted and non-targeted metabolomics revealed the differences among phytoplasma effector SAP54 homologs overexpressing *Arabidopsis* plants
Ling Dong (FSU, Theißen lab), [Abstract](#) page 58
25. Azelaic acid: A distress signal molecule
Priya Reddy (FSU, Oelmüller lab), [Abstract](#) page 59
26. Niche choice and niche conformance in grasshoppers, investigating the maintenance of colour polymorphisms
Lilian Cabon (FSU, Schielzeth lab), [Abstract](#) page 60
27. Early signaling events and physiological changes in plant-fungus-plant communication upon wounding and infection
Yelyzaveta Zhyr (Mithoefer lab), [Abstract](#) page 61
28. How bold, bright and nasty are poison frogs?
Francesca Protti-Sanchez (Rowland lab), [Abstract](#) page 62
29. Phloem-based metabolic changes in *Brassica nigra* linking its above- and belowground herbivores
Jessil Ann Pajar (FSU, Van Dam lab), [Abstract](#) page 63
30. Low apoplastic Na⁺ confers salinity tolerance upon Ca₂SiO₄ chemigation in *Zea mays* L. varieties under salt stress
Moniba Zahid Mahmood (Mithöfer lab, guest PhD researcher), [Abstract](#) page 64

Talks



Plenary Lecture 1

The Neuro-ecology of the large cabbage white butterfly: From receptors to the brain

Qi Wang¹, Stefan Bonestroo¹, Dimitri Peftuloglu¹, Liana Greenberg², Hans Smid¹, Marcel Dicke¹,
Nina Fatouros², [Alexander Haverkamp](mailto:alexander.haverkamp@wur.nl)¹

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Butterflies are among the best studied organisms with regards to their natural history, having fascinated both amateur as well as professional entomologists since Darwin's times. In spite of this wealth of information, rather little is known about the way in which butterflies perceive their environment. This lack of understanding becomes especially apparent when looking at the role of olfaction in adult females and caterpillars during host-plant evaluation and natural enemy avoidance.

In this project we took advantage of the relatively simple sensory system of the large cabbage white (*Pieris brassicae*) caterpillar to determine the role of olfaction during host-plant evaluation and the avoidance of natural enemies. We started out by generating antennal transcriptomes from both caterpillars and adult butterflies and by describing the general morphology of the caterpillar olfactory system. These analyses then formed the basis for the next step in which we knocked out the most highly expressed receptors as well as the ubiquitously expressed co-receptor *Orco*, using Crispr/Cas9. Knocking out *Orco* led to a general loss of neuronal activity in the insect antennae. In addition, caterpillars in which this co-receptor had been disrupted were unable to select a suitable host-plant and lacked responses to many host-plant volatiles. Surprisingly, *Orco* knock-out caterpillars were also found to be more susceptible to attacks by the parasitoid wasp *Cotesia glomerata*, demonstrating a role of olfaction in the enemy avoidance behavior of this caterpillar.

Besides *Orco*, we also succeed in knocking out several specific receptors among which *OR45b* was of special interest. This receptor was found to detect benzyl cyanide, which is not only an important odor for host-plant selection, but also an anti-aphrodisiac pheromone. The female receives it during mating and subsequently uses it to deter additional males. Interestingly, female butterflies lacking this receptor became more susceptible to another important natural enemy of *P. brassicae*, the egg parasitoid *Trichogramma evanescens*. This parasitoid is attracted to benzyl cyanide as it indicates the presence of a mated female, ready to lay eggs. These results suggest that *OR45b* might enable the butterfly to reduce the risk of egg parasitisation by *Trichogramma* wasps through a detection of dangerously high benzyl cyanide concentrations.

Using a genetic approach, our work gives some first insights into the importance of olfaction during the different life stages of a butterfly as well as into the specific role of individual receptors in the perception of its environment.

Plenary Lecture 2

Leaf beetle propagates a plant pathogen in exchange for pupal protection

Aileen Berasategui^{1,2,7,*}, Noa Breitenbach¹, Marleny García-Lozano¹, Ines Pons¹, Brigitte Sailer³, Christa Lanz⁴, Viterbo Rodríguez⁵, Katharina Hipp³, Nadine Ziemert², Donald Windsor⁶, Hassan Salem^{1,8,9*}

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Many insects rely on microbial protection in the early stages of their development. However, in contrast to symbiont-mediated defense of eggs and young instars, the role of microbes in safeguarding pupae remains relatively unexplored, despite the susceptibility of the immobile stage to antagonistic challenges. Today, I outline the importance of symbiosis in ensuring pupal protection by describing a mutualistic partnership between the ascomycete *Fusarium oxysporum* and *Chelymorpha alternans*, a leaf beetle. The symbiont rapidly proliferates at the onset of pupation, extensively and conspicuously coating *C. alternans* during metamorphosis. The fungus confers defense against predation as symbiont elimination results in reduced pupal survivorship. In exchange, eclosing beetles vector *F. oxysporum* to their host plants, resulting in a systemic infection. By causing wilt disease, the fungus retained its phytopathogenic capacity in light of its symbiosis with *C. alternans*. Despite possessing a relatively reduced genome, *F. oxysporum* encodes metabolic pathways that reflect its dual lifestyle as a plant pathogen and a defensive insect symbiont. These include virulence factors underlying plant colonization, along with mycotoxins that may contribute to the defensive biochemistry of the insect host. Collectively, our findings shed light on a mutualism predicated on pupal protection of an herbivorous beetle in exchange for symbiont dissemination and propagation.

Talk 1

Response plasticity of *Drosophila* olfactory sensory neurons

Lorena Halty de Leon¹, Eric Wiesel¹, Venkatesh Pal Mahadevan¹, Bill S. Hansson¹ and Dieter Wicher¹

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In insect olfaction, sensitization refers to the amplification of a weak olfactory signal when the stimulus is repeated within a specific time window. In the vinegar fly, *Drosophila melanogaster*, this occurs already at the periphery, at the level of olfactory sensory neurons (OSNs) located in the antenna. In our study, we investigate whether sensitization is a widespread property in a set of seven types of OSNs, as well as the mechanisms involved. First, we characterize and compare differences in spontaneous activity, response velocity and response dynamics among the selected OSN types. These express different receptors with distinct tuning properties and behavioral relevance. Second, we show that sensitization is not a general property. Among our selected OSN types, it occurs in those responding to more general food odors, while OSNs involved in very specific detection of highly specific ecological cues like pheromones and warning signals show no sensitization. Moreover, we show that mitochondria play an active role in sensitization by contributing to the increase in intracellular Ca^{2+} upon weak receptor activation. Thus, by using a combination of single sensillum recordings (SSR), calcium imaging and pharmacology, we widen the understanding of how the olfactory signal is processed at the periphery.

Talk 2

Exploring the olfactory neuroecology of *D. busckii*

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Spatial and temporal niches are often co-occupied by multiple animal species. Among drosophilid flies, a number of species have evolved to be human commensals, thereby often dependent on fruits, fungi and vegetables cultivated by humans. Here, we studied the olfactory neuroecology of one of the comparatively less studied human commensals; *Drosophila busckii*, representing the subgenus *Drosophila*. In contrast to the fruit-eating majority, this species is known to utilize rotting vegetables as food and oviposition substrate, thereby gaining a unique niche among drosophilids living with humans. A common feature of all substrates attractive to *D. busckii* is that, when rotting, they emit dimethyl disulfide (DMDS). We found that *D. busckii* is attracted to, eat from and oviposit on vegetable and fungal substrates emitting short chain volatile oligosulfides. The flies are also attracted to DMDS itself. To establish the olfactory background to this attraction we screened olfactory sensilla on the antenna of *D. busckii* using single sensillum recording technique. Among the olfactory sensory neurons (OSNs) present in the sensilla, we identified a physiological type narrowly tuned to oligosulfides and specifically to DMDS. DMDS, however, is known for its insecticidal activity and quickly killed most drosophilid flies tested. *D. busckii*, however, has evolved to tolerate high levels of DMDS, likely allowing it to exploit other food sources. Taken together, these features constitute an evolutionary strategy allowing *D. busckii* to utilize unique resources not exploited by competing drosophilids.

Talk 3

Learnt oviposition preference in the vinegar fly *Drosophila melanogaster*

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Female insects need to identify a suitable oviposition site to increase the survival chance of their offspring. Oviposition, however, is an adaptive process that is affected both by the available resources and by environmental conditions. Previous studies in *Drosophila* have shown that many factors such as e.g. parasitoids, pheromones from conspecific flies, the texture and hardness of the substrates, and visual cues can affect oviposition decision-making. Moreover, it has been shown in moths and butterflies, that experience with a given substrates can alter the oviposition preference which means that female insects are able to learn an oviposition site. In *Drosophila melanogaster* it is not known if female flies change their innate oviposition preference after learning. Therefore, we designed a single-fly assay for oviposition learning in *Drosophila* where we test the preferences of naïve and experienced flies under different conditions. Our results indicate that female flies alter their oviposition preference after experience significantly. Interestingly, after laying at least 4 eggs at the given substrate, flies increase their preference for this substrate. Even four days after the oviposition experience, the preference is still altered, suggesting the involvement of long-term memory in this learning task. We are currently investigating the nature of the reward that governs this learning in more detail.

Talk 4

Anatomical and cellular organisation of the olfactory system of *Locusta migratoria*

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The ability of the migratory locust, *Locusta migratoria*, to aggregate and form large swarms, categorises them as one of the most important agricultural pests in various regions across the world. This harmful phase in the life cycle of these insects highly relies on olfactory-guided behaviours. Olfactory cues are recognised by the odorant receptors of the olfactory sensory neurons (OSNs), gathered in hair-like structures, named sensilla, on two sensory appendages, the antennae and the palps of the mouthparts. In most insects, the primary olfactory centre, the antennal lobe (AL), is organised in a defined number of distinct globular structures, named glomeruli. Glomeruli are the synaptic site of three neuronal components: 1) the OSNs, 2) the projection neurons (PNs), the olfactory output of the AL to higher brain centres, and 3) the local interneurons (LNs), the circuitry neuromodulators. Stereotypically, a one-to-one wiring logic occurs here. That means that axonal projections of single OSNs and dendritic innervations of single PNs target, predominately, one glomerulus (uniglomerular OSNs/PNs). In contrast, locusts, which belong to the sub-order Caelifera of Orthopteran, display a particular organisation of AL with more than 1000 fused and singular micro-glomeruli wired by multiglomerular OSNs and PNs, making locust an interesting case of the evolutionary study of olfaction systems. In this on-going study, we aim to thoroughly characterise the anatomical and cellular architecture of the olfactory system in *Locusta migratoria*. By the use of anterograde staining of sensory neurons, immunohistochemistry and light scanning confocal microscopy, a 3D brain atlas of olfaction-related neuropiles is under construction. That includes in particular the AL, the lobus glomerulatus (LG) and the median crescent (MC). These three neuropiles form glomerular structures and receive sensory inputs of either exclusively olfactory or diverse chemosensory neurons. Furthermore, we successfully traced olfactory sensory neurons of identified sensilla into the antennal lobe, using single sensillum anterograde stainings, in order to unravel AL circuitry patterns, with respect to sensillum type (basiconics and trichoids) and topology. On the same grounds, focal injection staining of AL PNs is also employed.

Talk 5

Evolution of sex pheromone receptors in *Drosophila* flies

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Insects use olfaction for a diversity of inter- and intra-specific communications. In that way, sex pheromone perception plays an important role in speciation, allowing the attraction of conspecific individuals and repelling individuals from different species. Recent studies on the evolution of sex pheromones within the genus *Drosophila* identify a high diversity of sex-specific compounds that are mainly detected via olfaction. These studies also demonstrate that to keep the efficiency of inter- and intra-specific reproductive communication systems, a rapid and independent evolution of the sex pheromones and their associated olfactory channels is necessary. Using the transgenic expression of olfactory receptors with the empty neuron system in *D. melanogaster*, and the posterior measurement of electrophysiological responses through single sensillum recordings to previously identified sex pheromones, we are investigating how pheromone receptors coevolve to match the high diversity of sexual pheromones. We would also like to identify potential hotspots in the receptors undergoing rapid evolution.

Talk 6

Hijacking primary cell component for steroidal specialized metabolism

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Plants synthesize a vast repertoire of steroidal specialized metabolites. These include the well-known class of anti-nutritional steroidal glycoalkaloids (SGAs), which act as defensive chemicals in the *Solanaceae*, and the pharmacologically important and widespread steroidal saponins. *Solanum nigrum* (also known as black nightshade), a member of the *Solanaceae* family has been used in traditional medicine due to its anti-cancer, anti-fungal, anti-inflammatory, anti-diabetic and anti-bacterial properties. The co-existence of steroidal specialized metabolites, for example uttroside B, a steroidal saponin accumulating exclusively in leaves, and α -solasonine and α -solamargine, SGAs predominant in green and ripe berries in *S. nigrum* exemplify its potential in human health and nutrition. Both SGAs and steroidal saponins are synthesized from a shared precursor, cholesterol. Despite their importance, the corresponding biosynthetic pathways have not been fully elucidated yet, making it impossible to produce these valuable compounds in heterologous hosts. Here we present the characterization of a new GLYCOALKALOID METABOLISM15 (GAME15) protein– a missing link in *Solanum* steroidal specialized metabolites biosynthesis. We discovered that GAME15 is a result of an evolutionary molecular hijacking of the cell wall machinery that encompassed change of subcellular localization and loss of enzymatic activity. GAME15 evolved as an endoplasmic reticulum (ER) scaffold protein tethering other GAMEs from early steps of the pathway and enabling the production of both steroidal saponins and steroidal glycoalkaloids metabolites. Notably, the involvement of GAME15 in steroidal specialized metabolism was confirmed by complete loss of steroidal saponins and SGAs in *game15* knockout *S. nigrum* plants. Our findings provide a unique example of hijacking a core metabolism enzyme from the cellulose biosynthesis machinery that lost its activity during evolution and became a scaffold controlling the steroidal specialized metabolite biosynthesis in the genus *Solanum*.

Talk 7

Reinventing pathways: convergent evolution of specialized metabolites in flowering plants

Matilde Florean¹, Katrin Luck¹, Benke Hong¹, Sarah E. O'Connor¹, Tobias G. Köllner¹

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To cope with their sessile nature, plants synthesize an array of specialized metabolites that serve as defenses against a multitude of biotic and abiotic threats. Among specialized plant metabolites, benzoxazinoids (BXDs) form a class of indole-derived compounds with broad antimicrobial and antifeedant properties. Unlike many other specialized metabolites, which tend to be lineage-specific, BXDs occur sporadically in a number of distantly related plant families including Poaceae, Ranunculaceae, Lamiaceae, Acanthaceae and Apocynaceae, raising the question of whether BXD biosynthesis arose independently in different lineages. Decades of research in the Poaceae have led to the complete elucidation of the BXD metabolism in this monocot family. Here, the conversion of indole to BXDs is catalyzed by a series of evolutionarily related cytochrome P450s, a 2-oxoglutarate-dependent dioxygenase, and a methyltransferase. Despite the extensive knowledge of the pathway in monocotyledons, BXD biosynthesis in eudicotyledons is still unknown.

By using a metabolomic and transcriptomic-guided approach, we have succeeded in deciphering the BXD pathway in *Lamium galeobdolon* (Lamiaceae) and *Aphelandra squarrosa* (Acanthaceae). Interestingly, both species possess a dual-function flavin-containing monooxygenase that takes over the activity of two BXD cytochrome P450s from the Poaceae. In addition, we identified four evolutionarily unrelated cytochrome P450s, a 2-oxoglutarate-dependent dioxygenase and a O-methyltransferase involved in BXD formation in these species. Transient expression of the enzymes in *Nicotiana benthamiana* allowed us to fully reconstruct the BXDs pathway of *L. galeobdolon* and *A. squarrosa*. Our findings constitute the first characterization of BXD pathways in Eudicots. Moreover, the heterogeneous pool of identified BXD enzymes represents a remarkable example of metabolic plasticity, in which the ability to produce BXDs according to a similar chemical logic, but using a different set of metabolic enzymes, has independently evolved in distantly related plant families.

Talk 8

Uncovering the biosynthesis of gramine in barley and reporting a new UPLC-FLD method for its detection

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Modern cereal agriculture in Europe is experiencing novel challenges from multiple abiotic and biotic factors. Barley (*Hordeum vulgare*) is among the most cultivated crops, for feedstock and the production of beer, whereas lupin (*Lupinus spp.*) is cultivated as a green manure, as forage and its seeds are grown as an alternative to soybeans. Due to their economic and cultural importance, it is critical to study the biology and metabolism of these crops in the context of climate change and plant-herbivore interactions. Part of these plants' strong adaptivity to stresses is conferred by gramine. This indole alkaloid, found in vegetative tissues and seeds, has shown allelopathic activity against mammals, insects, bacteria, and fungi. Its defensive properties are an interesting trait in seeds production but a disadvantage in the forage for ruminants. With our study we aim to understand how gramine is biosynthesized in barley and how to efficiently detect its presence in plants.

We screened various accessions of barley to assess their gramine content. We analyzed the sequence data using the known gramine biosynthetic enzymes as a tool to discover further genes controlling its biosynthetic pathway. Candidate genes were expressed in yeast, *Nicotiana benthamiana* and *Arabidopsis thaliana*. We identified the enzyme responsible for the production of gramine's first stable intermediate, 3-aminomethylindole (AMI). Moreover, investigations were conducted with a new chromatographic method mediated by fluorescence detection, purposely developed for this project. The UPLC-FLD method produced calibration plots expressing linearity over the range 8-900 pmol/μL for gramine. The method was successfully applied in a study testing the range of expression of gramine and other analytes, such as hordenine, tryptophan, and tyramine in various accessions of barley, as well as in several species of lupins.

This project lays the foundation for future developments such as the manipulation of gramine levels for functional studies and innovative agricultural applications involving barley, lupin and other crops subjected to environmental challenges. Nevertheless, it provides an accessible and accurate chromatographic method for the separation and detection of this compound.

Talk 9

Exploring biosynthesis and regulation of triterpenoids in *Nicotiana attenuata* revealed a herbivore-defense function of pentacyclic triterpenes

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Triterpenes and their triterpenoid derivatives are widely distributed in plants as specialized metabolites. Many of the specialized metabolites and their function have been extensively studied in *Nicotiana attenuata*. However, the composition of the triterpene reservoir of *N. attenuata*, along with the associated ecological functions of these triterpenes, remains undiscovered. This study investigated the role that six oxidosqualene cyclases (OSCs), which are triterpene biosynthetic enzymes, play in regulation and herbivore defense. The enzymatic activity of these OSCs was studied by heterologous expression in *Nicotiana benthamiana*. Evaluations of the impact that these genes have on herbivore resistance were performed using OSCs-silenced *N. attenuata* plants.

NaOSC1 was shown to be a multifunctional enzyme with lupeol and β -amyrin synthesis activity, while both *NaOSC2* and *NaOSC3* had only β -amyrin synthesis activity. The *NaOSC1* are induced by jasmonate (JA) signals while *NaOSC2* are repressed, and the transcript accumulation also showed an opposite pattern in MYC2a/b-silenced plants. However, products of both enzymes, *NaOSC1* and *NaOSC2*, had defensive effects on *Manduca sexta*. Our results indicate that pentacyclic triterpenes with oleanane and lupinane as the main structures participate in herbivore defense in *N. attenuata* as constitutive and JA-inducible defense compounds.

Talk 10

Investigating the costs and benefits of a host plant-dependent chemical defense in a herbivorous insect

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Chemical defense compounds of plants play an important role in ecological networks by shaping interactions with herbivorous insects and their natural enemies. One mechanism through which plant defense compounds shape multitrophic interactions is sequestration, the accumulation plant defense compounds in the insect for protection from predators. We use the horseradish flea beetle (*Phyllotreta armoraciae*) as a model to understand the molecular basis and ecological consequences of insect sequestration strategies. This specialist herbivore sequesters glucosinolates from its brassicaceous host plants and uses endogenous myrosinase enzymes to convert them to toxic isothiocyanates for defense. We discovered that *P. armoraciae* possesses three different myrosinase genes with distinct ontogenetic expression patterns. These genes encode enzymes with different substrate preferences, which likely represent an adaptation to the divergent glucosinolate profiles of above- and belowground plant tissues that *Phyllotreta* adults and larvae use as a food source, respectively (Körnig et al., submitted). Interestingly, we additionally discovered natural genetic variation of myrosinase activity in *P. armoraciae*. Through haplotype-resolved genome sequencing, we found that the number of myrosinase genes encoded in the beetle genome varies between individuals. This gene copy number polymorphism influences the level of myrosinase activity in larvae but not in adults. The selection of beetle lines with larvae having high or low myrosinase activity allows us to investigate the costs and benefits associated with sequestered chemical defenses. For example, physiological costs of higher myrosinase activity may result from the detoxification of reactive isothiocyanates while having the benefit of a better protection against predators. To investigate possible costs, we currently compare several fitness parameters of beetles with high and low myrosinase activity under controlled conditions. To investigate benefits of this chemical defense, we determine the impact of high and low myrosinase activity on the ability to deter entomopathogenic nematodes, which are potential biological control agents of flea beetle pests in agricultural ecosystems. We hope that our results will contribute to the development of sustainable pest control strategies for flea beetle pests and to understanding how plant chemical defenses shape trophic networks.

Talk 11

Glucosinolates at the next trophic level: Effects of aphid-sequestered compounds on a ladybird predator

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Plants from the Brassicaceae family defend themselves against herbivores by producing glucosinolate and their toxic breakdown products. Some herbivores, such as the phloem-feeding cabbage aphid *Brevicoryne brassicae*, have in turn evolved mechanisms to cope with these plant toxins, including sequestration for their own defense against predators. Such herbivore self-defense mechanisms constitute a possible route through which non-target organisms at higher trophic levels, such as the aphids' natural enemies, are exposed to plant defense compounds. Within the Brassicaceae plant family, different species often display major differences in glucosinolate composition, and this results in different sequestration profiles in the aphid body. Our knowledge of how plant chemical diversity mediates such multitrophic interactions is scarce, especially for interactions involving phloem-feeding herbivores. I am studying how an herbivore predator, the ladybird beetle, metabolizes the glucosinolate toxins of its aphid prey. Investigations on different ladybird species (Coccinellidae) that appear to be more or less resistant to the glucosinolates of *Brevicoryne brassicae* will allow us to better understand the impact of plant chemical defense diversity in a multitrophic context.

Talk 12

Gut symbiont transmission in bark beetles

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The Eurasian spruce bark beetle (*Ips typographus* L., Coleoptera: Curculionidae: Scolytinae) is the most destructive forest pest in Europe. The rising temperatures have shortened the beetle's life cycle, as well as increased the stress on the host tree, the Norway spruce (*Picea abies* L., Pinaceae). These conditions have favored the occurrence of mass outbreaks, where the beetle populations transition from attacking weak or fallen trees to infesting and killing healthy ones. *Ips typographus* associates with several ophiostomatoid fungi that help them overcome the tree's chemical defenses. While the roles of these fungal ectosymbionts have been previously studied, the involvement of other microbial partners in the beetles' ecological success is unclear. The aim of this study was characterize the bacterial and fungal communities of *Ips typographus* linked to the natural history of this insect.

We compared the gut microbiome of wild-collected and lab-reared *Ips typographus* across all its life stages. To do so, we characterized the bacterial and fungal communities using amplicon sequencing of the 16S and Internal Transcribed Spacer (ITS) ribosomal RNA genes. I complemented the data by isolating and identifying bacteria from adults, larvae and oral secretions from females. Additionally, I used phloem sandwiches to observe beetle behavior and record possible mechanisms of symbiont acquisition and transmission.

The results showed that the bacterial communities of *Ips typographus* were dominated by genera belonging to the Gammaproteobacteria (*Erwinia*, *Pseudoxanthomonas*, *Pseudomonas*, *Ochrobactrum*, *Izhakiella*). The fungal communities were mainly composed by yeasts belonging to the Saccharomycetales order (*Ogataea*, *Kuraishia*, *Peterozyma*, *Saccharomycopsis*, *Wickerhamomyces*, *Yamadazyma*). The fungal and bacterial taxa associated to the insects were distinct from those associated to the bark surrounding the beetle galleries.

A stable core of microbes shared by all the life stages, but distinct from those associated to the surrounding bark, suggests that bark beetles pass their gut symbionts from one generation to the next. Maternal inoculation via the deposition of oral secretions next to newly laid eggs, as well as coprophagy in immature imagoes, may be mechanisms to transfer symbionts to other individuals. The persistence of a core microbiome could be a sign of the existence of a functional role in the beetle's nutrition. I will further investigate these results with a mechanistic approach to confirm the transmission mechanisms and explore the functional roles of the core taxa *in vitro* and *in vivo*.

Talk 13

Protecting symbiotic bacteria: a novel role of host-derived cuticular hydrocarbons

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In symbiotic systems host and symbiont must overcome many biotic and abiotic challenges for the successful transmission of the association across generations. One essential barrier that symbionts must surmount is their hosts' immune defences. Our knowledge of host adaptations to protect their symbionts against their own immune system in extracellular symbiotic systems is scarce, although such adaptations are likely widespread in nature. Using a combination of bioassays, RNA sequencing and proteomics in the beewolf wasp-*Streptomyces* symbiosis, we show how an insect host combines enzymatic and physicochemical mechanisms to secure its bacterial symbiont's survival when exposed to high concentrations of nitric oxide produced by the beewolf egg (an extension of the immune system of the host used to sanitize the brood cell). Remarkably, beewolf wasps embalm their *Streptomyces* symbiotic cells with a layer of linear saturated and unsaturated hydrocarbons that confers an effective barrier preventing the diffusion of NO, an important function not previously described for cuticular hydrocarbons, which play other key roles in communication and desiccation avoidance in insects. These results exemplify the complexity of host adaptations to protect symbiotic bacteria against own immune effectors securing the transmission and providing stability to an ancient defensive symbiosis.

Talk 14

Inhibition of the shikimate pathway by glyphosate disrupts the stability of symbiosis and impairs host fitness across diverse grain pest beetles

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Glyphosate is the most widely used herbicide globally and acts by inhibition of the shikimate pathway that is lacking in animals but present in plants and many microbes. However, insects are ubiquitously associated with nutritional microbes that are localised in the gut or intracellularly in bacteriomes; and that may be sensitive to glyphosate. Some studies have begun to shed light on the detrimental effects of glyphosate on insect hosts via their associated microorganisms, especially when the host relies on microbe-supplemented shikimate products that are important for cuticle formation. Nevertheless, we still lack an understanding of how generalized the impacts of glyphosate are in insects. Thus, we here aimed to unravel how the herbicide affects host fitness in 10 species of grain pest beetles, encompassing six different families. These families include taxa with intracellular symbionts that encode the shikimate pathway and those without known shikimate-producing symbionts. We first show that glyphosate generally reduces the titers of associated microbes and this manifests in significantly impaired cuticle melanisation in glyphosate-treated beetles. Furthermore, the perturbation of microbial partners by glyphosate was reflected in an overall decline in host reproductive output, with fewer or no adult progeny emerging from glyphosate treatments when compared to the controls. Additionally, species with fungal symbionts that do not encode the shikimate pathway were not negatively affected by glyphosate. Surprisingly, the most dramatic effect was observed in the two Tenebrionid species that lack intracellular symbionts but where larvae nevertheless completely failed to enter metamorphosis in the glyphosate treatment. An analysis of the community composition will reveal if there are changes in the gut microbial consortia of these two species, which might explain the failure to pupate. Given the widespread taxonomic distribution of nutritional symbioses, our findings suggest that glyphosate may put many insect populations at risk by inhibition of the shikimate pathway via their associated microbes, an effect that extends even beyond specialized intracellular symbionts to general gut communities.

Talk 15

Symbiont interactions and chemical mediators in a defensive symbiosis

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Symbiotic associations between insects and microbes are widespread and the benefits provided by the symbionts can be crucial for the survival of the host. Many insects only engage in mutualisms with particular bacterial species but a community of different taxa can also occur in a host. Although host-symbiont relations are often well studied, the interactions between different symbionts and their influence on the composition and functionality of a community remain poorly understood. Maintaining a symbiont homeostasis is important for keeping a symbiosis functional. Symbiotic functions comprise for example nutritional supplementation of poor diets, pheromone production or defense against antagonists.

In *Lagria villosa* beetles (Coleoptera: Tenebrionidae) we find such a defensive symbiosis in which bacteria protect beetle offspring from fungal infestation. The microbial community of field-collected *L. villosa* is dominated by *Burkholderia gladioli*, but other bacteria like *Variovorax*, *Acinetobacter* and *Luteibacter* can also be found consistently across all life stages. Within *B. gladioli* we can find multiple symbiont strains. While *B. gladioli* Lv-StB is the most abundant strain in nature, it is lost after a few generations of lab rearing. *B. gladioli* Lv-StB has an eroded genome and cultivation attempts have not been successful so far. In contrast, *B. gladioli* Lv-StA is less abundant in nature but is culturable *in vitro*. Both strains produce different bioactive secondary metabolites. While Lv-StB mainly produces the potent antifungal compound lagriamide, Lv-StA has a broader spectrum of secondary metabolites producing for example lagriene, gladiofungin, sinapigladioside, cayoyncin, burriogladin, haerreogladin and icosalide, some of which could also influence colonization success. To investigate the effect of co-occurrence of multiple *Burkholderia* strains on colonization success we utilize *in vivo* assays. We are also interested in the impact of single secondary metabolites produced by Lv-StA on the defense. Therefore, single knockout mutants were generated and tested for activity against different fungi. With our findings, we hope to contribute to the overall understanding of symbiont-symbiont interactions in multipartite symbiotic systems.

Talk 16

Nematodes dampen division of labour in ants

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In social groups, disease risk is not distributed evenly across group members. Individual behaviour is a key source of variation in infection risk, yet its effects are difficult to separate from those of other factors. Here, we combine long-term epidemiological experiments and automated tracking in clonal raider ant colonies, where behavioural individuality emerges among identical workers. We find that: 1) division of labour determines the distribution of parasitic nematodes (*Diploscapter*) among hosts, showing that differences in infection can emerge from behavioural variation alone, 2) infections affect colony social organisation by causing infected workers to stay in the nest. By disproportionately infecting some workers and shifting their spatial distribution, infections reduce division of labour and increase spatial overlap between hosts, which is expected to facilitate parasite transmission. Thus, division of labour, a defining feature of many societies, not only shapes infection risk and distribution but can also be modified by parasites.

Talk 17

Impact of a novel *Trichoderma* strain on the host's physiological response to salt stress

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Salt stress is one of the major environmental factors that limits plant productivity. To mount an effective response to cope with salt stress, plants rely on the salt overly sensitive (SOS) pathway. This includes SOS1, SOS2 and SOS3 proteins that are crucial for the maintenance of ion homeostasis and the *sos1* mutants are hypersensitive to salt stress. Symbiotic association of plants with beneficial microbes can increase the tolerance of plants to various abiotic stresses. One such versatile endophytic plant symbiont is *Trichoderma* spp. that can protect plants against salt stress. However, the molecular mechanism by which it mitigates the damage caused by salt stress remains elusive. In this study, we examined the effect of novel *Trichoderma* strain on the growth of *glabara1* (*gl1*) and salt overly sensitive (*sos1*) mutants in *Arabidopsis thaliana* under salt stress conditions. Our results demonstrate that several plant growth parameters such as fresh weight, quantum efficiency of photosystem II and chlorophyll content was improved in *Trichoderma* inoculated plants under salt stress. Moreover, *Trichoderma* colonized plants showed enhanced transcript levels of genes involved in Na⁺ and K⁺ homeostasis. Accumulating evidence exists that plants can adapt to salinity stress through regulation of phytohormones and it would be interesting to find out how *Trichoderma* influences the plant hormone levels. Taken together, our results will unveil the beneficial role of *Trichoderma* spp. in improving the antioxidant and osmo-protective status of plants under salt stress.

Talk 18

The evolution of a dual function component in a noctuid moth

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Sexual signals are key components in mate finding and mate choice and their evolution is likely to play an important role in speciation. Sexual attraction through sex pheromones is best studied in moths, where females emit species-specific pheromone blends. This species-specificity stems from the presence/absence and ratios of the different components. For example, the two closely related noctuid moth species *Heliothis subflexa* and *Heliothis virescens* produce similar sex pheromone blends, with one major difference: acetate esters. Only *H. subflexa* females produce acetate esters in their sex pheromone blend which play a dual function: attracting conspecific males, while repelling the sympatrically occurring species, *H. virescens*. Moreover, *H. subflexa* females produce high acetate ester levels when *H. virescens* are present, but low in their absence.

Discovering 1) the genetic basis of variation in acetate esters and 2) possible fitness costs of acetate production, will help us understand how such a dual function has evolved. Previous genetic analyses have identified two major QTLs underlying acetate esters variation. In this study, we identified a few candidate genes that are involved in acetate esters variation in both QTLs. In addition, we selected for high and low levels of acetate esters in *H. subflexa* and investigated how this affected the pheromone blend. Finally, we investigated whether the production of high acetate ester levels comes with a cost, by rearing the two selection lines under food stress conditions. Our results indicate that 1) intra- and inter-specific variation in acetate ester levels can be explained by a combination of shared and unique genetic factors and 2) high levels of acetate esters come with a fitness cost under stressful conditions.

Talk 19

Color morph variation in the meadow grasshopper *Pseudochorthippus parallelus* is controlled by a few genetic loci

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The green-brown polymorphism found in polyneopteran insects is one of the most pervasive color polymorphisms found in any group of animals. This color dimorphism is widespread in 30% of all European orthopterans and 45% of East African acridid grasshoppers. It is distributed across most habitats but is particularly common in moist and alpine grasslands. There are inconsistent findings on whether these color polymorphisms originated from the environmental and/or genetic factors. We here report on breeding experiments with the meadow grasshopper *Pseudochorthippus parallelus*, which is polymorphic for the presence and distribution of green body parts. We scored phenotypes of 4,293 offspring. We find that the rearing environment did not affect the polymorphism, and that morph ratios did not differ between the sexes. Furthermore, we scored color features like leg and wing color, wing stripe, pied pattern, and pink variants. We find strong evidence for genetic determination both for the presence/absence of green and its distribution. Our findings contribute to the growing gap between the straightforward genetic determination of green-brown polymorphisms in Gomphocerinae species and environmental determination in other grasshopper subfamilies (Oedipodinae, Cyrtacanthacridinae, and Acridinae). The initial findings point to one of a few loci with allele dominance that allow for the presence of green pigmentation in three of the four Gomphocerinae species studied thus far. This adds support to the notion that brown people differ from green people due to homozygosity for loss-of-function alleles that inhibit the formation or deposition of pigments that contribute to green color.

Talk 20

“Why so (un)predictable?” Correlates of (un)predictability in escape behavior in a grasshopper

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Throughout life, animals are faced with innumerable challenges and individuals differ from each other in how they respond to such challenges. In the context of escaping from predators, prey species strategies can be particularly manifold. Besides inter-individual differences in average escape behavior, there is an intraindividual dimension of variability, as individuals may differ in how variably they respond to different predator encounters. This dimension of animal personality has been poorly explored due to methodological constraints, but recently gained some of the attention that it deserves. With the development of sophisticated analysis, it is now possible to access correlates of the inter-individual behavioral variation, at the same time as accounting for intra-individual differences. The extremes of escape strategies can be classified as unpredictable, or as predictable, but efficient in the use of a straight, long-distance escape path. The first seems particularly advantageous in complex habitats where unpredictable individuals can hope to find hiding cover, while the second might be more efficient in unstructured habitats. However, the "chosen" strategy might also critically depend on the costs in terms of energy requirements. Using quantitative genetic tools, and the steppe grasshopper *Chorthippus dorsatus* as model organism, we quantified the relative role of environmental and genetic influences on both aspects of behavioral variability. In order to access the phenotypic flexibility in (un)predictability in response to environmental conditions, we scored escape behavior of individuals raised in cages with poor versus enriched environments. We also tested if immune-challenged individuals that are in impaired overall condition become more or less predictable in behavior. And, finally, we followed individuals throughout life to study the ontogeny of (un)predictability in behavior across multiple life stages.

Talk 21

The role of FLC-like genes in *Aethionema arabicum*

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The genus *Aethionema* possesses a phylogenetically informative position at the base of the Brassicaceae family. Developing both dehiscent and indehiscent fruits on the same plant *Ae. arabicum* features an interesting dimorphism that makes it a valuable model species to study the developmental mechanisms controlling trait evolution in the Brassicaceae. Previous studies have drawn a complex picture concerning the way in which the ratio of dehiscent to indehiscent fruits depends on the growth habit of the plant. The *FLOWERING LOCUS C* (*FLC*) gene and closely related paralogs (*MAF* genes) are key regulators of flowering time in Brassicaceae members. In addition to their role in regulating flowering time via thermosensory response, the *FLC*-like genes have also been found to be involved in growth habit in other Brassicaceae members. We hypothesize, therefore, that *FLC*-like genes might have a major impact on growth habit, fruit dimorphism and ecological adaptations in *Ae. arabicum*. Our quantitative expressional studies revealed the similar pattern of expression of orthologue of *FLC* like in *Arabidopsis thaliana*, *AearFLC* found to be expressing more in vegetative parts like roots, leaves, apices than fruits and negligible in flowers and floral buds. Towards that goal, we are generating loss-of-function mutants for *AearFLC* employing CRISPR-Cas9 and overexpressing lines using a constitutive promoter in *Ae. arabicum* via an *in vitro* regeneration system. To obtain additional information about the function of *FLC* in *Ae. arabicum* we make use of the detailed knowledge about the interaction of *FLC* with other transcription factors and target genes in *Arabidopsis thaliana* and *Arabis alpina*. Using *in silico* genome comparisons, the yeast two-hybrid system (Y2H) and electrophoretic mobility shift assays (EMSA) we are studying similarities and differences in protein-protein and protein-DNA interactions involving *FLC* of the three Brassicaceae species.

Talk 22

Selection pressure by specialist and generalist insect herbivores leads to optimal constitutive plant defence - a mathematical model

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Plants of the Brassicaceae family have the glucosinolate-myrosinase defense system, jointly active against herbivory. Glucosinolates (GLS) are hydrolyzed by myrosinase to produce isothiocyanate products as soon as herbivory begins. Isothiocyanates exert detrimental effects on the feeding insect herbivores. However, constitutive GLS defense is observed to occur at levels that do not deter all insects from feeding, and that prompts the question about why Brassicaceae plants have not evolved a high level of constitutive defense. The answer may lie in the contrasting relationship between plant defense and host plant preference of specialist and generalist herbivores (two different groups of insects).

One of the reasons plants are in this dilemma is that they do not really know what kind of herbivore will attack them in any given year, and thus have to be prepared for different possibilities. On the one hand, GLS increase the susceptibility of a plant to specialist insects because specialists are attracted to plants with a high GLS content and are capable of coping with the toxin. On the other hand, generalist insects are deterred by the plant GLS content. Although plant GLS can attract the natural enemies (predators and parasitoids) of these herbivores, they can reduce the herbivore pressure to some extent only. So, plants can be overrun by specialists if GLS content is too high, whereas generalists can invade the plants if GLS content is too low.

Since a host plant can be attacked by different groups of insects at the same time, an optimal constitutive plant defense can minimize the overall herbivore pressure. To explain it theoretically, we represent the contrasting host selection behavior of insect herbivores and, in addition, the emergence of their natural enemies by a non-autonomous ordinary differential equation model, where the independent variable is the plant GLS concentration. From the model, we quantify the optimal amount of GLS, which minimize the total herbivore (specialists and generalists) pressure. That quite successfully explains the evolution of constitutive defense in plants from the perspective of optimality theory.

Talk 23

Desert ants build their own landmarks in a featureless environment

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The desert ant, *Cataglyphis fortis*, inhabits the harsh and arid saltpans of Tunisia, where food resources are limited. These ants are known to employ individual foraging strategies and primarily rely on path integration to navigate back to their nest. However, path integration can be prone to errors, and its accuracy and reliability decrease with increasing foraging distance. To compensate for these limitations, desert ants use both visual and olfactory cues to locate their nest entrance. In this study, we investigate the homing accuracy and mortality rate of desert ants after prolonged foraging runs and identify a strategy used to cope with the high risk of getting lost. Our findings reveal that ant nests in the center of the saltpan have taller nest hills than those located closer to the visually structured border of the saltpan. Additionally, ants returning to nests located in the center of the saltpan rely heavily on the presence of a nest hill while those returning to a nest close to the landmark-rich border do not. Experiments with or without artificial landmarks revealed that the removal of the nest hill induces strong efforts into rebuilding the hill when no landmarks are present, while the presence of artificial landmarks is sufficient to suppress hill building. Obviously, the desert ant purposively builds its own landmark in a featureless environment, by manipulating the height of its nest, which ultimately increases its chances of successful homing and survival in the harsh saltpan environment.

Posters



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Poster 1

To oviposit or not to oviposit: a host selection dilemma by *Manduca sexta*

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Lepidopterans evaluate olfactory, gustatory and visual cues before selecting an oviposition site. A single oviposition experience with a given plant can tune the tobacco hawkmoth's (*Manduca sexta*, Sphingidae) preference towards ovipositing on the same plant again, which could be an adaptive trait under unpredictable conditions. We worked with two well-known host plants (Solanaceae) of the hawkmoth *M. sexta* and a less studied one (Martyniaceae), which possesses similar physical and nutritional characteristics as the other two. We asked, whether *M. sexta* females after one oviposition experience with a given plant results later in a preference for the same plant species, when the moth can choose between individual leaves of the three plants as egg laying sites. Our results show that moths after experiencing a given solanaceous plant (*Datura wrightii* or *Nicotiana attenuata*), later oviposit on the leaves of these plants and in total lay more eggs on these leaves. Only a few of the naïve moths oviposited on the presented *Proboscidea parviflora*, so that whether or not experience with that drives later preference cannot yet be concluded. However, along these experiments, we observed that older (but still naïve) moths touched the leaves of *Proboscidea* plants with the proboscis and drummed their first two pair of legs (drumming behavior) for less time than younger moths before ovipositing during first experience. Maybe the eagerness to oviposit increases with age and increases the moth's willingness to oviposit on less preferred plants. With slow motion video we clarify the behavioral components of oviposition behavior in *M. sexta* and our data furthermore gives insight into the ecological trade-offs of host selection.

Poster 2

Effect of hybridization on the plant metabolome

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Hybridization is an important evolutionary force, estimated to shape 30-70% of our modern plant species. However, little is known about the effect interspecific hybridization has on plant phytochemistry. A good model for hybridization studies is the genus *Baccharis* (Asteraceae), as it contains more than 30 putative interspecific hybrid taxa. Chemically still under-characterized, *Baccharis* is commonly used in folk medicine, and known for producing multiple compounds with interesting pharmacological activities. By using untargeted metabolomics, we characterized the chemodiversity of a *Baccharis* hybrid complex *in order to expand our* current understanding of hybridizations' chemical, ecological, and evolutionary consequences. To do so, we collected more than 700 parental and hybrid plant samples along the Chilean coastal area for methanol extraction and an untargeted LC-MS (qTOF) analysis. Chromatograms were annotated using SIRIUS and visualized through principal component analysis (PCA) and molecular networking. After applying k-means clustering, each subgroup's chemodiversity was characterized using diversity indexes. We found that *the different parental species* showed a clearly distinct metabolic profile, rich in flavonoids and diterpenoids. PCA reported the hybrid species as an intermediate chemotype between parental species while k-means clustering helped identifying the hybrid distribution across the different locations. The hybrids showed a higher chemodiversity (as measured by richness, and Shanon index) than the pure parental species, suggesting the presence of novel hybrid chemistry. One of the parental species showed a distinct chemical pattern corresponding to the north and south subpopulations, which suggests a recent speciation event occurring through the longitudinal axis.

Poster 3

Adaptive plasticity in specialists and generalists

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Increased anthropogenic activities have led to strong detrimental effect on the environment and climate change. Rise in global temperature and gaseous pollutants disturb the ecological interactions and the insect biodiversity. A recent study from our lab showed that levels of ozone found in rural areas disrupt the innate attraction of the tobacco hawkmoth *Manduca sexta* to the odour of one of its preferred flowers (Cook et al. 2020). However, this study showed that moths are able to learn ozone-altered floral odours through reward learning. Therefore, we speculate that insects such as Drosophilids might also possess similar ability in coping with modified odours through anthropogenic pollutants. We therefore investigate the effect of ozonated and/or polluted odours on the behaviour of “generalists” (Drosophilids that live in a wide ecological niche) and “specialists” (Drosophilids that live in a particular place). We hypothesize that “generalists” could adapt better than “specialists” in an increasingly polluted atmosphere. For instance, *D.sechellia*, a specialist Drosophilid, has adapted peripheral receptors and neural circuits in the brain allowing the insect to feed on noni fruit which other drosophilids strongly repel. These specialized changes which are beneficial to thrive in a specific niche might be the cause for specialists like *D.sechellia* to be less adaptive to increasing levels of pollutants in the environment. The objective of the project is to study the adaptations and plasticity of various drosophilids in response to pollutants. Through behavioral experiments, we found that *D.melanogaster* flies upon ozonation exhibit a decreased response to attractive odours and an increased aversion to aversive odors. Further functional imaging and electrophysiological experiments will reveal the neuronal mechanism behind the observed modified odour perception in ozonated flies at a functional and behavioral level. Furthermore, we aim to analyze the learning abilities of the different drosophilids to adapt to modified (i.e. ozonated) food odors.

Poster 4

Sex and position in the canopy determine the chemodiversity of the leaves in old-growth black poplar (*Populus nigra*) trees

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The crowns of woody plant species often reach enormous heights and diameters. Therefore, the abiotic and biotic conditions within the canopy of mature trees can be very heterogeneous, but so far, not much is known about the effect of season and vertical stratification within tree canopies on leaf chemodiversity. Furthermore, there may be sexual dimorphism in the chemical traits of dioecious trees. The aim of this study was to investigate spatiotemporal trajectories in leaf chemistry of mature male and female black poplar trees growing in a natural floodplain forest in Northeastern Germany. Leaves from five different heights of the canopy of nine trees were harvested and analyzed using targeted and non-targeted LC-MS/MS analytics. Our data show that both sex and position within the tree canopies affect the chemical composition and diversity of black poplar leaves. Our study highlights the importance of vertical stratification and sex on the chemical diversity of *P. nigra*.

Poster 5

Dissecting odor mixture interactions in the fly brain

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In the natural environment, odors are often blends of many compounds at different concentrations. A question arises then, how does the olfactory system encode different mixtures of odors? The vinegar fly represents hereby a premier model system to study olfactory processing and mixture coding since the olfactory circuitry exhibits a stereotyped architecture which is similar to its mammalian counterpart, but which is less complex and highly tractable as well as susceptible to genetic manipulations. In a previous paper, we demonstrated that mixtures of odors having opposing hedonic valences (at certain ratios) are encoded and processed by a mixture-specific activation of projection neurons, the output neurons of the fly antennal lobe (Mohamed et al., 2019). This mixture code is maintained through lateral inhibition in an anisotropic manner initiated between glomeruli encoding opposing odor valences. We found that the activity of patchy and sparse local interneurons mediate this specific form of mixture inhibition. The goal of this project is to study the calcium dynamics of these patchy local interneurons in response to diverse odor mixtures, at the level of their pre- and post-synaptic activities. We will investigate both the population dynamics, as well as activities at the single neuron level using two-photon functional imaging of the fly antennal lobe using SPARC.

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Poster 6

Chemical signaling in alga-parasite interactions in aquatic ecosystems

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In open oceans, microalgae serve as primary producers of the food web and live in complex interactions with microorganisms. The algal host and its cohort of symbionts create a biomolecular network with exchanged chemical cues, coined as the algal holobiont. The algal host cells are using specialized metabolic and behavioural strategies to thrive despite biotic and abiotic stresses, such as pathogens and heat waves. For instance, the microalgae from the genus *Coscinodiscus* produce beta-carboline alkaloids during parasite infection by marine oomycetes, but their production and ecological role remains unexplored in diatoms. To date, few studies have focused on the algal holobiont and the role of alkaloids in host cell regulation during stress.

Here, we investigate the cell metabolism using metabolomics and we are analyzing the alkaloid production in diatoms in response to the presence of oomycete parasites and temperature increase. Our research aims to elucidate the identity of these metabolites in the host-symbiont interactions and their function in stress responses. We will also study the chemical defence strategies in algal hosts during parasite attacks using non-contact dual cultivation and metabolomics.

Poster 7

From land to water and back again: Neuroanatomical adaptations of the olfactory system in Hemiptera insects

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Hemiptera constitutes one of the largest and most diverse orders of insects. Members of the sub-order of Heteropteran, the so-called 'true bugs', have successfully adapted to a wide range of terrestrial and aquatic ecosystems with a cosmopolitan distribution. Phylogenetic studies have estimated the hemipteran transition towards aquatic habitats in the early Permian (296Ma). Under environmental changes and ecological pressures, evolutionary diversification gave rise to a lineage of secondarily aquatic true bugs (Infraorder Nepomorpha), which developed a variety of novel morphological, physiological and behavioural adaptations to different freshwater environments, including shortened antennae, swimming legs, specialised underwater breathing mechanisms and feeding strategies. An exception to the aquatic lifestyle of Nepomorpha is the superfamily of Ochteroidea which has secondarily adapted back to land. The remaining ones (Corixoidea, Nepoidea, Notonectoidea, and Naucoroidea) are entirely aquatic. Based on anatomical observations in Notonectoidea species, aquatic true bugs have been considered anosmic, due to the absent of brain structures typical for terrestrial insects i.e., the lack of antennal lobe (AL; primary olfactory centre) with distinct glomeruli, and of mushroom body (MB) calyces (a secondary olfactory centre). However, recent studies of the antennal sensilla in nepomorphan species, revealed the presence of chemoreceptive uni-porous and multi-porous sensilla, typically responsible for gustation and olfaction. With the present study we intend to establish the neuroanatomical layout of the olfactory system in three particular species, the backswimmer *Notonecta glauca* (Notonectidae) and the creeping bug *Ilyocoris cimicoides* (Naucoridae) and the velvety shore bug *Ochterus marginatus* (Ochteridae), with emphasis on their transitions from land-to-water (*Notonecta*, *Ilyocoris*) and water-to-land (*Ochterus*) and their respective adaptations. Anterograde tracing of afferent sensory neurons, histochemical techniques and light scanning confocal microscopy enable a morphometric assessment and comparison of the structures of interest among the studied species. Our observations confirm the absence of obvious MB calyces in all three species, but they all have complex multi-lobed MB peduncles and a group of MB intrinsic cells. Though, we contest previous reports of an absence of a glomerular AL in *Notonecta* species. We identified a reduced AL neuropile composed by roughly 30 distinct but partially fused glomerular complexes in *N. glauca*, predominantly innervated by multi-glomerular input sensory neurons. A similar AL organisation was observed in *I. cimicoides*. In the land-adapted *O. marginatus* we found a relatively larger AL and more distinct glomeruli. Currently we employ focal injection staining for identification of the axonal tracts that connect the sensory neuropils with the central brain.

Poster 8

Evolution of fruit scent in Madagascar's figs

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Fruits have evolved to be attractive to seed dispersers, and fruit traits evolved in response to selection pressures by frugivores. Fruit scent has evolved as a signal for frugivores, signaling ripeness and potentially fruit quality. However, it is still unclear how and what parts of fruit scent have evolved as signals for frugivores and what information they contain. Unlike most chemical classes, aliphatic esters are found to be concentrated in species that communicate with seed dispersers via chemical cues and only in ripe fruits. Some evidence and theoretical basis have indicated a positive correlation with sugar content. This means that there might be a link between chemical signals and where chemical coevolution is expected for fruit dispersed in lemurs. Our research aims to identify whether aliphatic esters are indeed an honest signal for fruit quality and test whether it is an adaptation acquired by plants. We test this hypothesis on a model system of up to 20 fig species (*Ficus* spp; MORACEAE) growing in Madagascar. With collections of fruit and leaf, we intend to (1) establish an ecological network, (2) reconstruct phylogeny, (3) identify the link between chemical signal and reward by using thermo desorption gas chromatography-mass spectrometry (TD-GCMS) and high-performance liquid chromatography (HPLC) to test whether it is the unique link between species and dispersal ecology, and (4) to sequence alcohol acyltransferase (AAT) to check if the selection regimes corresponding to the dispersal mode.

Poster 9

Evolution of the olfactory system in feeding *versus* non-feeding moths

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Nocturnal animals like moths largely rely on their sense of smell when searching for food sources. Most hawkmoths, for example, feed on floral nectar and are strongly attracted by the scent of night-blooming plants. Recently, our lab could show that hawkmoths fail to feed on floral nectar when their sense of smell was impaired by mutagenesis of the main family of chemosensory receptors, so-called odorant receptors (ORs), showing that ORs are crucial for foraging. However, there are also Lepidopteran species closely related to nectar-feeding hawkmoths that have reduced mouthparts and do not feed as adults. In our study, we asked if this essential difference in the moths' life history has an impact on their olfactory system. We aimed to answer this question at three different levels by investigating feeding (n=7) and non-feeding (n=7) species belonging to the same taxonomic group (Bombycoidea). First, we studied the anatomy of the antennal lobe, the first processing center of olfactory information, to test if non-feeding species might have less or differently organized functional subunits, so-called olfactory glomeruli. Our initial findings show that non-feeding species have on average five glomeruli less than their feeding relatives. Second, using the correlation between the total number of olfactory glomeruli and receptor genes we estimated the success of our approach by comparing the expression of ORs at the moths' antenna and proboscis using Illumina-based transcriptome sequencing. We hypothesized that non-feeding species have a reduced number of ORs. Last, we will use *in vivo* calcium imaging of the antennal lobe to reveal potential differences between non-feeding and feeding moth species regarding neuronal coding of typical floral scent compounds and seven headspaces obtained from plant species visited by hawkmoths. With this molecular, anatomical and physiological comparison, we hope to identify olfactory pathways essential for the detection of floral food sources.

Poster 10

The interface of host-symbionts interaction

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Many insects have obtained evolutionary success by establishing close associations with microbial organisms. As a result, most bacterial symbionts that reside inside insects have co-evolved to be adapted to their specialized niche by genome size reduction and maintaining limited metabolic capacities which benefit host fitness. Among nutrient supplementary symbionts, some bacteria in beetles complement tyrosine synthesis via the shikimate pathway and support host cuticle formation which confers resistance against desiccation, predation and pathogens. Larger grain borers *Prostephanus truncatus* (Coleoptera: Bostrichidae) are grain pests of stored maize and harbor ancient symbiotic Bacteroidota bacteria in bacteriomes that diverged into multiple separate strains. These strains are anticipated to have partitioned genetic basis of the shikimate pathway and ribosomal proteins, requiring exchange of small metabolites or macromolecules. However, it is unclear what evolutionary driver resulted in this uneconomic microbial association in the beetles and how it is retained. Therefore, this project aims to reveal the distribution and interactions of multiple strains of nutrient supplementary endosymbionts in *P.truncatus* throughout each life stage. With fluorescence in situ hybridization (FISH) approaches, I will be able to snapshot how multiple bacterial cells collaborate for the shikimate pathway in a bacteriome and how host beetles regulate them over generations. By integrating these observations with bacterial genome assembly and phylogenetic analyses, this project aims to better understand the physiological integration and evolutionary history of the endosymbiont lineage fragmentation.

Poster 11

Manipulating the sterol metabolism in tomato for the production of vitamin D₃

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Vitamin D₃ is a vital nutrient required by the human body to support healthy bone growth and maintenance. Ultraviolet radiation of the sterol 7-dehydrocholesterol (pro-vitamin D₃) produces vitamin D₃ in human skin. Deficiency of vitamin D₃ increases risk of osteoporosis, hypertension, autoimmune disease, infectious disease, diabetes and cancer. Dietary supplementation of vitamin D₃ is required for human health, but natural sources of vitamin D₃ are limited and strictly, animal based. Therefore, there is a great need to find alternative, sustainable sources of vitamin D₃. Plants harbor an enormous reservoir of diverse steroidal molecules and in principle, could be a source of vitamin D₃. The precursor of vitamin D₃, 7-dehydrocholesterol, is also the immediate precursor for cholesterol biosynthesis in plants. Since most plants produce cholesterol in very low amounts, 7-dehydrocholesterol levels are low as well. However, certain *Solanaceae* family plants (e.g. tomato) accumulate naturally high levels of cholesterol, yet possess low amounts of 7-DHC, thus limiting the efficient production of vitamin D₃. Using pathway knowledge and combinations of state-of-the-art synthetic biology, gene editing and unconventional metabolic engineering approaches, here we developed two strategies to alter endogenous sterol biosynthetic pathways in tomato plants to enhance the accumulation of 7-dehydrocholesterol and further vitamin D₃.

Poster 12

Creating a genetically tractable symbiosis: experimental symbiont replacement in grain pest beetles

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Symbiosis with microorganisms is a crucial factor in insect diversification as it can enable insect hosts to exploit new ecological niches. Unfortunately, the intimacy of many symbioses hampers research on this topic: The lack of experimental tractability of host and/or symbiont commonly prevents genetic manipulation of insect associated microbes, which is important to understand how different symbiont genotypes can affect host fitness. Sawtoothed grain beetles (*Oryzaephilus surinamensis*, Silvanidae) harbor intracellular *Shikimatogenerans silvanidophilus* bacteria that supply their insect host with tyrosine precursors, vital for the synthesis and melanization of the host's cuticle. While *S. silvanidophilus* remains unculturable, the ability to retain symbiont-free *O. surinamensis* opens opportunities for experimental manipulation: Replacing native *S. silvanidophilus* symbionts with a genetically tractable bacterium could allow for the genetic engineering of this symbiosis. *Sodalis praecaptivus* is a free-living bacterium that is closely related to several insect associated *Sodalis* species. Because it is I) culturable and amenable to genetic engineering and II) able to infect insect hosts like tsetse flies and weevils, we considered *S. praecaptivus* a suitable candidate to act as a novel symbiont in *Oryzaephilus*. To assess its infection capabilities, we injected two fluorescently labeled strains of *S. praecaptivus* (the wild-type and a tyrosine overproducing strain) into the hemolymph of reproducing females and monitored survival and infection status of the beetles and their offspring. Using live-imaging and microscopy approaches, we show that both wild-type and tyrosine overproducing *S. praecaptivus* could establish systemic infections in the beetle host and were vertically transmitted from mother to offspring. We also present first insights into our attempts to unravel the interactions between the novel symbiont and *O. surinamensis*, focusing on how *Sodalis* influences cuticle development as well as investigating putative host control over symbiont titers. Our findings represent the foundation of establishing a genetically tractable model symbiosis that will allow for studying host-symbiont interactions in a nascent mutualism.

Poster 13

Development of single-cell mass spectrometry method for the study of the medicinal plant *Catharanthus roseus*

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State-of-the-art analytical techniques for single cell metabolomics are still hampered by many challenges due to low sample volume, rapid changes in the metabolome and the need to measure a structurally diverse group of metabolites. Therefore, more work is needed to develop sensitive, robust and reliable methods to profile metabolites in single cells. We have been developing a metabolomics method for simultaneously targeted and untargeted analysis of isolated plant single cells (protoplasts) allowing identification and quantification of several metabolites. The medicinal plant *Catharanthus roseus*, which produces anti-cancer drug vinblastine and vincristine was chosen for this case study. Here, we designed a process in which a high precision microfluidic cell picking robot was used to collect protoplasts prepared from *C. roseus* leaves from a Sieviewell™ device. Protoplasts were transferred to 96-well plates, lysed and directly analyzed by an optimized UPLC-HRMS method. Quantification of metabolites within the cells was possible using external calibration. This method provides medium throughput and includes chromatographic separation prior to mass spectrometry, greatly enhancing accurate assignment and absolute quantification of metabolites. We are aiming to expand its applications to protoplasts from other *C. roseus* organs, such as roots and flowers and to other plant species. We believe this method will provide a valuable tool for scientists in the fields of plant science and biotechnology.

Poster 14

Endosymbionts contribution to the cuticle synthesis of a pest beetle.

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Beetles are distinguished by their hard cuticle that protects them against physical injuries, predators, parasites, and environmental stresses. Tyrosine is a key metabolite in cuticle synthesis and its modifications (sclerotization and melanization), but insects have to obtain it from their diets as they lack the capability to synthesise it on their own. However, several beetle families harbour bacterial endosymbionts that can biosynthesize the precursor of tyrosine, thus contributing to the cuticle synthesis, pigmentation and hardening in nutritionally unbalanced environments. *Dinoderus porcellus* is a food storage pest beetle, and harbours two closely related bacterial endosymbionts localized in separated organs. The symbiont *Candidatus* Shikimatogenerans bostrichidophilus retained mainly genes involved in the shikimate pathway to produce the precursor of Tyrosine, while the second symbiont, *Candidatus* Bostrichicola ureolyticus, retained genes involved in urea recycling and the biosynthesis of peptidoglycan, lysine, methionine, and vitamins.

In this study, we investigate the functionality and integration of the endosymbionts' biosynthetic pathways. We specifically test whether the recycled nitrogen from urea is incorporated into the tyrosine and the host cuticle, resulting in an exchange of metabolites between the host and the two endosymbionts. We quantified urea, and uric acid in different larvae stages and adults, to be able to estimate the amount of labeled ¹⁵N-urea and ¹⁵N-uric acid to inject in the last instar larvae and quantify labeled ¹⁵N-amino acids in the hemolymph and the cuticle of young adults.

We also investigate the contributions of both endosymbionts to the host physiology, and their regulation, by manipulating the beetle's diet. After supplementing insects' diets with single amino acids, selective antibiotics, or pharmacological inhibitors, we evaluated the impact on symbionts and host, by monitoring both symbionts' titers and measuring the host cuticle's thickness and darkness (melanisation). Correlating diet nutrition, symbiont titer and cuticle measurements will allow to draw conclusions about the contribution of endosymbionts on the host physiology, but also to get insights into possible regulatory mechanisms involved in this tripartite interaction.

Poster 15

Metabolism of spruce phenolic defense compounds by a symbiotic fungus of bark beetles

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Norway spruce (*Picea abies*) is susceptible to attack by the European spruce bark beetle, *Ips typographus*, and one of its symbiotic fungal associates, *Endoconidiophora polonica*. The natural interactions of this bark beetle and its fungal associates on spruce hosts are complex. The symbionts are introduced into the host tree during bark beetle invasion. It has been hypothesized that infestation of *E. polonica* could enhance the development of the bark beetle by metabolizing the defensive chemicals of the tree. A major group of phenylpropanoid phenolics called stilbenes are assumed to protect Norway spruce trees from herbivore attack and microbial infection. However, the mechanisms that the bark beetle and its fungal associates might apply to avoid stilbene toxicity and deterrence remain unclear. In this study, we aim to reveal how symbiotic fungus *E. polonica* metabolizes spruce stilbenes. The metabolites of the predominant spruce stilbenes, including piceid, resveratrol, astringin and piceatannol, are being detected by untargeted metabolomics analysis, and further quantified by targeted analyses via HPLC-MS/MS. Furthermore, the genes and proteins of *E. polonica* involved in these processes are being explored. We hope to find out how *E. polonica* overcomes stilbene defenses and colonizes its spruce host, and how this affects bark beetle colonization.

Poster 16

Dynamics and mechanisms aiding symbiont colonization in a darkling beetle

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Many insects are associated with bacterial symbionts that provide nutrients, defend against pathogens or detoxify harmful compounds. Among these, extracellular symbionts usually occur in different locations across the host life-cycle. While entering into the host symbiotic organs, symbionts encounter physiochemical or immunological challenges from the host, or compete with other microbes. Microbial molecular factors that help overcome these challenges are known only from studies on pathogens or a few mutualistic symbioses. Here, we investigated the dynamics and molecular mechanisms of colonization in *Burkholderia* symbionts in *Lagria villosa* beetles. In this association, bacteria produce antifungal compounds that protect the beetle from fungal pathogens. Adult female beetles host one or multiple *Burkholderia* strains in specialized glands that are accessory to the reproductive system, and they smear the bacteria onto the egg surface during oviposition. Experiments with one of the strains show that microbe-mediated mechanisms help symbionts colonize the three dorsal surface cuticular invaginations in the first-instar larva. The larvae are capable of acquiring symbionts horizontally from the environment, however this appears to be less efficient in the later larval stages. Using transposon-insertion directed sequencing, we identified a number of potential colonization factors, including some involved in stress response, motility, and iron-regulation. A comparison of genomic capabilities between coexisting symbiont strains leads us to speculate that the mechanisms for colonization differ among the strains of *Burkholderia* associated with the same host. Thus, the *Lagria-Burkholderia* symbiosis not only presents a genetically tractable system to study the molecular basis of symbiosis establishment, but also provides a rare opportunity to compare colonization strategies among closely related bacteria and assess their interactions in a multipartite symbiosis.

Poster 17

An evolved gamma-aminobutyric acid aminotransferase drives steroidal glycoalkaloid biosynthesis in *Solanum*

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Steroidal glycoalkaloids (SGAs) are defence specialized metabolites produced by hundreds of *Solanum* species including staple vegetable crops, such as tomato (*Solanum lycopersicum*) and potato (*S. tuberosum*). Apart from their undesirable, anti-nutritional properties, many steroidal glycoalkaloids display advantageous pharmacological activities. In recent years, several steps of the SGA biosynthetic pathway starting from cholesterol precursor leading to production of SGAs scaffold and glycosylated forms have been elucidated to a large extent. Incorporation of nitrogen to steroidal scaffold is a key step in SGA biosynthesis. This reaction was hypothesized to occur through transamination of steroidal backbone catalysed by gamma-aminobutyric acid aminotransferase (GABA-T) enzymes. In core metabolism, GABA-Ts (e.g. GABA1) act in the GABA shunt pathway, and are known to transfer an amino group from GABA to an acceptor molecule, for example, pyruvate. Here, we show that GLYCOALKALOID METABOLISM12 (GAME12), a cytosolic enzyme evolved from GABA-Ts of core metabolism, is involved in biosynthesis of nitrogen containing steroidal alkaloid aglycone in different *Solanum* plants. While GABA1 is targeted to the mitochondria and GABA3 to the chloroplast, GAME12 is localized to the cytoplasm. Using a wild *Solanum* species, *S. nigrum*, which produces steroidal saponins exclusively in the leaves, we show that overexpression of *GAME12* from tomato results in metabolic shift that enables *De Novo* production of SGAs in *S. nigrum* transgenic leaves. We also demonstrate that the cytosolic sub-cellular localisation is crucial for the GAME12 activity, and that the remaining GABA-T isoforms do not contribute to the SGA biosynthesis in *Solanum* plants. We also investigated the architecture of the active site of the GABA-Ts including GAME12 and identified a structural motif necessary for transamination activity in the SGA biosynthesis. Our results highlight the central role of GAME12 as a gateway enzyme in the SGAs biosynthetic pathway, and demonstrate that 'hijacking' of core metabolism GABA shunt proteins and modification of its subcellular location as well as its active site is crucial in the *Solanum* plants for introducing the nitrogen into steroidal specialized metabolic pathways.

Poster 18

Impact of the microbiome on *Drosophila melanogaster* cold tolerance

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More than ten thousand years ago, *Drosophila melanogaster*, an ectotherm organism, was able to colonize new habitats in temperate regions while originating from a sub-Saharan climate. The remarkable success of the expansion the fruit fly abiotic niche is dependent on thermal tolerance mechanisms, which allow the insect to survive fluctuating temperature regimes by tolerating modifications of its metabolism and in the case of cold tolerance recovering from lethal injuries induced by a cold shock. These mechanisms are highly dependent on the insect nutritional status and survival to colder viable is known to be diet specific. In *Drosophila melanogaster*, gut microbes are acquired via food intake and have been described to participate in a multitude of metabolic functions via provisioning essential precursors and nutrients thus having an impact on the nutritional status of its host. This suggests that gut microbial communities could participate directly or indirectly in cold tolerance mechanisms in *Drosophila melanogaster*.

Combining performance assays, with gnotobiotic flies, and 16S amplicon sequencing, we aim to identify microbial candidates involved in cold tolerance within the viable temperature range and across sub-optimal temperatures as well as the mechanisms involved in such processes. Here we show that gut microbes are rescuing cold induced delay in development rate and reduced reproductive success enabled by a combined active host-microbe interaction and a passive nutritional supplementation. We also show that gut microbes are able to rescue its host from lethal cold injuries. In particular, *Lactobacillus plantarum*, one of the persistent known gut microbes, seems to be a key actor to freeze tolerance since its sole presence is able to rescue the *D.melanogaster*.

Further studies aiming at understanding the mechanistic basis of such interaction are needed and necessary to predict survival of natural populations of microbe associated insects beyond our fruit fly laboratory strain, especially in the context of global change.

Poster 19

The formation of iridoid trail pheromones in the Argentine ant *Linepithema humile*

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Iridoids are cyclopentane[c]-pyran monoterpenoids that are widely distributed in plants and insects. They are mainly considered as defense compounds, but also act as pheromones in insects. While the biosynthesis of iridoids in plants has been intensively studied, little is known about how insects produce these compounds. In this project, we aim to study the formation of iridoids in the Argentine ant (*Linepithema humile*), an ant species native to South America but now invasive in many Mediterranean climates. *L. humile* workers have been described to produce the iridoids dolichodial and iridomyrmecin as trail pheromones. By comparing the transcriptome of the abdomen, the site of iridoid production, with that of the head and thorax of adult workers, we identified genes potentially involved in iridoid biosynthesis. We are now characterizing the candidate genes by heterologous expression in different host systems followed by in vitro enzyme assays. We have already shown that one of the candidate genes, which is highly expressed in the abdomen of worker ants, encodes a functional isopentenyl diphosphate synthase capable of producing geranyl diphosphate, the precursor of iridoid formation in plants and insects.

Poster 20

Effect of nematode infections on nestmate-recognition in clonal raider ants

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Parasites can influence virtually every aspect of host biology, including behavior. Parasites can influence host behavior over a broad range, from manipulations that increase disease spread to host behavioral responses that reduce pathogen spread. One among the several parasites that infect ants are nematodes that belong to the genus *Diploscapter*. *Diploscapter* infections in ants have been reported since the 19th century. However, the mechanisms of infection, the effect of these infections on host physiology and behavior, and the dynamics of infection spread remain largely unexplored.

We have established an experimental system with *Diploscapter* infecting the pharyngeal gland (PG) of the clonal raider ant *Ooceraea biroi*. The PG is involved in the storage and spread of cuticular hydrocarbons among nestmates. Cuticular hydrocarbons play a central role in communication within ant societies as they are the basis for the formation of a “colony odor”, which the workers then use for nestmate recognition. Previous work shows differences in the cuticular hydrocarbon profiles of uninfected and *Diploscapter*-infected ants. However, it is currently unknown whether infections influence the ants’ nestmate recognition abilities, and if so what the consequences on behavior and parasite transmission are. In this project, I aim to answer how the nematode infections influence host cuticular hydrocarbon profiles and the ability of the ants to discriminate nestmates from non-nestmates, and how this in turn influences the social behavior, parasite transmission, and colony fitness.

Poster 21

Toxicity of cardenolides from *Asclepias curassavica*

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Milkweed–herbivore systems are characterised by cardenolide chemical defences and specialised herbivore adaptations such as physiological target site insensitivity. Cardenolide defences in milkweeds can vary in terms of the total concentration, differences in the polarity of individual cardenolides via the various glycoside groups, and in the steroidal cores by the presence of hydroxyl or carbonyl groups than can contribute to the molecule's reactivity. The variability in cardenolide defences is suggested to characterise the plant's response to natural selection and adaptation of resistant herbivores, and represent phenotype-matching between defensive and offensive traits resulting from coevolution. Here we test this idea by comparing the cardenolide composition of seeds of *Asclepias curassavica* and those sequestered by nymphs and adults of the specialised seed herbivore *Oncopeltus fasciatus*. We combine this with tests of the inhibitory capacity of a subset cardenolides isolated and purified from *A. curassavica* seeds against the Na⁺/K⁺-ATPase of *O. fasciatus*, and a non-adapted insect (*Drosophila melanogaster*), and compared this to the highly sensitive porcine Na⁺/K⁺-ATPase. Among the five most abundant cardenolides present in milkweed seeds glucopyranosyl frugoside, glucopyranosyl gofruside, and glucopyranosyl calotropin were significantly more abundant in the seeds than the adults and nymphs, which preferentially sequestered the deglycosidated compounds. The most abundant compound, glucopyranosyl frugoside, was also the most inhibitory for *O. fasciatus*, but *Oncopeltus* was significantly more tolerant of all compounds compared to *D. melanogaster* and the highly sensitive porcine enzyme. Our results add to the evidence that *O. fasciatus* selectively sequester individual cardenolides from their *Asclepias* host plants, and that this is related to the availability and structure of the cardenolides in the seeds, and to the tolerance of the insect to the ingested cardenolides.

Poster 22

saxA as public good? - Rethinking the role of pathogens in leaf microbiomes

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Leaf-colonizing microorganisms are exposed to extensive repertoires of plant secondary metabolites, but it is unclear how this shapes colonization at a community level. Although the leaves look healthy, bacterial microbiomes commonly include opportunistic pathogenic bacteria. We hypothesize that these bacteria can live in balance as commensals but that their special adaptations, for example manipulating the production of plant secondary metabolites, may shape bacterial community assembly. We investigate this using *Arabidopsis thaliana* and its secondary metabolites glucosinolates (GLS) as model system. Upon contact with myrosinase enzymes GLS are hydrolyzed to form diverse breakdown products which play roles in plant defense against herbivores and pathogenic microbes. Isothiocyanates (ITCs) are the best-studied antimicrobial breakdown products and have been suggested to be present in healthy leaves at levels relevant for colonizing bacteria. Some pathogens have evolved resistance to these compounds, which is encoded by so-called *sax* genes (*survival in Arabidopsis extracts*). *saxA* encodes a hydrolase, which degrades ITCs and thereby promotes virulence of both bacterial and fungal leaf pathogens. We already found that 4MSOB-ITC, which is the main GLS breakdown product in *Arabidopsis thaliana* Col-0, reduces growth of commensal bacterial strains *in-vitro*, but opportunistic pathogens like *Pseudomonas* and *Xanthomonas* were more resistant. *Pseudomonas syringae* 3D9 has *saxA*, degrades 4MSOB-ITC *in-vitro* and co-occurs in our local wild *A. thaliana* populations with ITC-sensitive commensal strains. Thus, we hypothesize that *saxA* might act as public good by degrading ITCs, which not only benefits the pathogen but also ITC-sensitive commensals. To test this, we designed co-culturing chambers which allow diffusion of 4MSOB-ITC across a 0.2µm filter membrane but do not allow bacterial cells to cross. Preliminary results showed higher growth of the commensal *Plantibacter* sp. 2H11-2 and reduction of 4MSOB-ITC in both wells when co-cultured with *P. syringae* 3D9 in one well. We currently aim to knock out *saxA* in *P. syringae* 3D9 to investigate the effect of *Pseudomonas*-produced SaxA on diverse commensal strains *in-vitro* and *in-vivo* during leaf colonization. This will be accomplished by using amplicon sequencing to characterize how a synthetic bacterial community with either WT or Δ *saxA* *P. syringae* colonizes healthy and mechanically damaged leaves.

Poster 23

Identification of plant 17,20- α -desmolases

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Progestogens and androgens are steroid hormones that play an important role in the development of mammals. They were also discovered in plants, and it is postulated that steroidogenesis in plants occurs similarly to that in mammals. Cyp17A1 is a human 17,20-desmolase, which is responsible for turning progestogens into androgens and therefore plays an important role in steroidogenesis. The influence of progestogens and androgens on plants has previously been studied, however it is not known if plants can turn progestogens into androgens. In this study we used a new Ultra High-Performance Liquid Chromatography and Mass-Spectrometry based method to verify that a wide range of plants could turn progestogens into androgens. Measurements of steroid contents were conducted on the green algae *Chlamydomonas reinhardtii*, the moss *Physcomitrella patens*, the gymnosperm *Larix decidua*, the monocotyledon *Hordeum vulgare* and the dicotyledon *Arabidopsis thaliana*. Bioinformatic analysis showed a strong similarity between the structures of the Cyp706-family of enzymes and Cyp17A1. Steroid measurements are being conducted on Cyp706-Knock Out lines of *A. thaliana* after supplementing progesterone in order to identify potential plant 17,20-desmolases. The role of steroid hormones in defense against fungal infections is also being investigated via infection of *A. thaliana* and the duckweed *Spirodella polyrrhiza* with the fungus *Alternaria brassicicola*. Our results show that the conversion of progestogens into androgens is phylogenetically conserved in plants and increase the understanding of steroidogenesis pathways in plants.

Poster 24

Targeted and non-targeted metabolomics revealed the differences among *phytoplasma* effector SAP54 homologs overexpressing *Arabidopsis* plants

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Gram-positive bacteria *Candidatus phytoplasma* are pathogenic and detrimental to diverse plant species all over the world. Phyllody symptom, characteristic of transformation of floral organs into leaf-like structures, is frequently observed in many phytoplasma-infected plants and thus pose a challenge to the agriculture and ecological environment. Phytoplasma effector SAP54, originally identified in phytoplasma strain AYWB, was demonstrated to induce phyllody in *Arabidopsis thaliana*. Later, more SAP54 homologs were found in different phytoplasmas and most could induce phyllody except for one which contains only two-amino acid difference from the phyllody-inducing SAP54s. To the aim of understanding how genetic diversity of different SAP54 homologs affects symptomatic variation, we examined metabolome of leaf tissue of seven different SAP54 overexpressing *Arabidopsis* genotypes and one GFP-control genotype by using targeted and non-targeted metabolomics. Results revealed significant differences in the metabolomic profiles among these genotypes. Taken together, this study will provide new insights into phytoplasma manipulation of plant metabolic programming by effectors and support control strategies in future agriculture.

Poster 25

Azelaic acid: A distress signal molecule

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Plant – microbial interaction is a complex web of interactions. Plants in their natural environment face drought, salinity, temperature, and other biotic stresses. After local perception of stress, the information is spread within the plant body to activate appropriate responses in systemic tissues or organs. Azelaic acid (AzA), a C9 oxylipin (“HOOC (CH₂)₇ COOH”), plays a prominent role in this scenario. The oxylipin AzA is synthesized in the plastid outer membrane and triggers immunity through an AZI1/EARLI1/MPK3/6-dependent pathway; but the traverse of AzA in the plant system remains a mystery. Upon fungal or bacterial elicitor application to roots, AzA accumulates in the leaves and triggers the expression of the gene of its receptor AZI, a lipid transfer protein. I investigate how the *Azi1* gene expression can be stimulated in the systemic tissue and intend to decipher its traverse pathway.

Poster 26

Niche choice and niche conformance in grasshoppers, investigating the maintenance of colour polymorphisms

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Homochromy, i.e. the matching of an organism's colour to that of its local background, is widespread in grasshoppers. Achieving homochromy enables grasshoppers to be cryptic in their environment. However such crypticity in grasshoppers is context-dependent. Indeed, grasshoppers are living in heterogeneous habitats presenting a mosaic of green (fresh grass) and brown (bare soil and dry grass) patches. Colour polymorphisms are widespread in grasshoppers, especially the green-brown polymorphism. Some species exhibit fully-green and fully-brown individuals while other species additionally have half-green half-brown morphs. Grasshoppers also differ in melanisation within colour morphs, resulting in a great individual-level diversity in terms of colour phenotypes. Both discrete (green-brown) and continuous (melanisation) polymorphisms can have behavioural and physiological consequences. Indeed, *(i)* to achieve homochromy grasshoppers have to preferentially settle in the local environment that is best suited to their phenotype, a mechanism called matching habitat choice; *(ii)* the colour-mediated thermoregulation hypothesis predicts that darker individuals (here fully-brown and/or melanistic individuals) have a thermoregulatory advantage. Yet, the green-brown polymorphism in grasshoppers is still poorly studied and among the available literature on this topic, no paper has addressed the question of melanisation beyond the altitudinal context. We will study behavioural, physiological and phenological consequences of individualised body colour in grasshoppers, aiming at bringing insights on how discrete and continuous polymorphisms are maintained in populations. To do so, we will contrast niche chooser and niche conformer species. Niche choosers are the species for which colour morphs are genetically determined while niche conformers are species able to change colour among life stages. To address this issue, we will set up laboratory experiments to determine whether individuals depending on their body colour differ or not in terms of thermal niche, temporal niche, microhabitat choice and mate choice. We will also collect field data on seasonal activity across three years to assess if individualised body colour in grasshoppers leads to different activity windows through the year.

Poster 27

Early signaling events and physiological changes in plant-fungus-plant communication upon wounding and infection

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For all living organisms, survival in any environment requires exchanges of energy, matter, and information. The exchange of the latter might be considered as communication and a key player in surviving strategies. Nowadays, the existence of plant-plant communication is widely accepted in the scientific community. It was repeatedly confirmed that plants are able to recognize signals and respond to them (e.g., signals released from aggressors as well as those from stressed neighbours informing about upcoming or existing threats).

One of the most widespread mechanisms in aboveground plant-plant communication is the release of volatile compounds. Belowground communication, in contrast, might be mediated by non-volatile molecules or by employing the help of mutualistic fungi (e.g., mycorrhizae). In general, symbiosis with fungi can provide minerals and water to plants, but also promote the transport of nutrients or active molecules. Moreover, during colonization, fungi can prime plants making them more resilient to (a)biotic stress. However, there is not much knowledge about early signaling events and physiological changes in belowground plant-plant communication due to wounding or infection in the presence of a symbiotic association with an endophytic fungus.

Our goals are to find out whether primary and secondary metabolites are affected by the presence or absence of fungal connections between plants in the first hours post-infection and whether changes in cytosolic Ca^{2+} levels ($[\text{Ca}^{2+}]_{\text{cyt}}$) might play a role in interplant signal mediation.

In order to reach these goals, we developed a method for the $[\text{Ca}^{2+}]_{\text{cyt}}$ response analysis in *Arabidopsis thaliana* plants expressing GCaMP3 (the Ca^{2+} -reporter) and connected by *Serendipita indica* (formerly *Piriformospora indica*) mycelial bridge. We quantified the amino acid, sugar, and phytohormone contents in the mentioned system upon *Alternaria brassicae* infection at different time points (24, 48 and 120 hs) and analysed $[\text{Ca}^{2+}]_{\text{cyt}}$ changes upon wounding in both, the infected/treated donor and the non-infected/treated receiver plants.

From our current results, it is clear that colonisation by *S. indica* not only affects the primary and secondary metabolism of plants, but also the development of *A. brassicae* infection. Importantly, we found no significant differences in the measured parameters between connected/unconnected by endophytic fungus receivers in the presence of donor infection or wounding.

Poster 28

How bold, bright and nasty are poison frogs?

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Predation is a selective pressure shaping many aspects of organisms. Therefore, prey species may evolve a variety of defence strategies to avoid predation, such as defensive behaviours, structures that may cause harm to the predators, cryptic coloration that prevents their detection by predators or warning signals that warn predators it would be costly to attack them, a strategy known as aposematism. Such antipredator strategies can be categorized on a continuum, from conspicuous, unpalatable or toxic (aposematic species) to camouflaged, harmless (cryptic). Behaviour should evolve to complement other antipredator traits: aposematic animals should be bold, visible, and slow moving, whereas cryptic animals should be timid, hide more, and move quickly. However, systematic data testing this assumption are limited, especially within vertebrate prey and within closely related species that share similar habitats. In this study, we aim to quantify antipredator defences in six species of dendrobatid poison frogs to test whether antipredator behaviour reliably fits within an aposematic-cryptic continuum. We assessed frogs' behaviour through three experimental settings: latency to exit a refuge, time budget and movement trajectory in an arena, and escape distance from a bird model. In addition, we measured frogs' colouration to assess conspicuousness and sampled skin secretions through UHPLC/GCMS in order to obtain toxin profiles. So far, behavioural results do not show strong support for the evolution of predictable behavioural strategies along an aposematic-cryptic gradient. Instead, the behaviour of each species appears to coincide with its specific microhabitat (vegetative cover, refuges, and resources). Ongoing work to characterize the alkaloids in skin secretions of these frogs, as well as conspicuousness for potential predators, will help to contextualize better these behavioural results. Overall, our results will aid to disentangle co-evolution among antipredator traits.

Poster 29

Phloem-based metabolic changes in *Brassica nigra* linking its above- and belowground herbivores

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Plant defense response can create a signaling network that allows for the interaction of herbivores bounded by time and space. One example is the indirect effect of root herbivory by plant parasitic nematodes (PPN) to the phloem-feeding aphid, *Brevicoryne brassicae*. With *Brassica nigra* as the common host, an increase in *B. brassicae* population was observed in root-knot nematode (RKN, *Meloidogyne* spp.)-infected plants compared to the uninfected control plants. The opposite effect was observed in aphids feeding on plants previously infected with the root lesion nematode (RLN), *Pratylenchus penetrans*. While this was attributed to nematode-induced phytohormonal signaling interfering with aphid-induced defense responses, many possible mechanisms are still yet unexplored. Moreover, these experiments were on single nematode infections, whereas in nature plants interact with many nematode species simultaneously.

Here we addressed the question how single and dual nematode infections belowground may affect aphid performance aboveground. We found that *P. penetrans* lowered aphid survival and co-infection of nematodes (RKN+RLN) decreased aphid population growth. Using untargeted metabolomics, we explored the possible plant-mediated mechanisms underlying the observed aphid responses. By tapping into the phloem of the common host plant, we found that plant defense and nutritional quality played a varying role on the effects of PPN on the performance of aphids. Our preliminary data shows what changes in the phloem metabolome are induced specifically by each nematode species and how this may have consequential effect to the performance aphids aboveground.

Poster 30

Low apoplastic Na⁺ confers salinity tolerance upon Ca₂SiO₄ chemigation in *Zea mays* L. varieties under salt stress

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Salinity is known to have a greater impact on shoot growth than root growth. Na⁺ buildup in plant tissue under salt stress has been proposed as one of the main issues that cause growth inhibition and affect crop growth. However, the evidence for apoplastic Na⁺ buildup, its relation to salinity tolerance especially in crops, and the role of silicon in the Na⁺ accumulation at the subcellular level are still enigmatic. The current study focuses on the accumulation of Na⁺ in the apoplast, symplast, and total shoot of younger and older leaves of two maize varieties (Iqbal as salt-tolerant and Jalal as salt-sensitive) using hydroponic culture along with silicon (1 mM) supplementation under short-term salinity stress. Subcellular ions analysis indicated that silicon nutrition decreased Na⁺ concentration more in older and younger leaves of the 'Iqbal' as compared to the 'Jalal' maize variety. The addition of silicon under 100 mM NaCl treatment resulted in considerable improvement in fresh biomass, relative water content, chlorophyll content, and concentration of important ions (*i.e.*, Ca²⁺, Mg²⁺, and K⁺). In the current study, soluble Na⁺ concentration in the apoplast of older leaves was found significantly maximum (36.1 mM) in the Jalal variety under 100 mM NaCl treatment, which was 42.4% higher when compared to the Na⁺ concentration in the Iqbal variety under the same treatment. Our findings suggest that the enrichment of calcium silicate increased *Zea mays* L. growth by higher water contents, reduced concentration of Na⁺ in the leaf apoplast, and intercellular homeostasis, which might as a result ameliorate the adverse effects of salt stress.