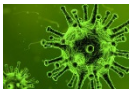
IMPRS
Exploration of Ecological Interactions with Molecular and Chemical Techniques

19th IMPRS Symposium

June 30 – July 01, 2020



Register here until June 29 (noon):

https://zoom.us/meeting/register/tJEtduCugDopE9BC7oMZOfOpG8mZAkvq9T83

Guest speaker:
MPI-CE alumna:
Dr. Silke Allmann

Please join us for:

- ***17 PhD talks***
- ***8 virtual coffee groups***
- ***3 best talk awards***

Program

Tuesday, June 30th, 2020

08:30	**Arrival** *Join Zoom Meeting (register first!)* *https://zoom.us/j/95019661394?pwd=NGlEb2tqYjJrQnBBYkg2SHgzUVRpQT09*
08:35	**Welcome** *Claudia Voelckel*
08:45	**New arrivals and defended theses in 2019/2020** *Jonathan Gershenzon*
09:00	**Plenary Lecture** **Scent and scentability: Understanding specificity in plant volatile signaling** *Dr. Silke Allmann, Department of Plant Physiology, University of Amsterdam,* p. 6 *chair: Jonathan Gershenzon*
09:45	***Coffee break(out)s:*** 1 - PhD rep meet & greet (Emily Puckett) 2 - Introducing the MPI-CE podcast channel: Synthetic Opinion (Sin Prelic) 3 - PhD almost done - what's next? (Karin Groten) 4 – Publishing and thesis issues (Kirsten Heinrich, Angela Günter) 5 - How do I market my research and fulfill my PR requirements? (Angela Overmeyer) 6 – IMPRS faculty meeting (Jonathan Gershenzon) 7 – Just coffee and chat (for non-PhDs and non-IMPRS faculty) 8 – Guest speaker Q&A (Silke Allmann)

	1st talk session *chair: Elisabeth Adam*
"Fungal volatiles & plant enzymes"	
10:15	1 - Volatile emission from endophytic fungi colonizing black poplar leaves *Christin Uhe (GER), p. 7*
10:30	2 - Arabidopsis response to a S-containing fungal volatile from *M. hyalina* under sulfur deficiency *Yu-Heng Tseng (Oelmüller), p. 8*
10:45	3 - Aggregation of β-glucosidases in herbivore-damaged maize *Diana Radisch (GER), p. 9*
11:00	4 - Evolution of enzyme pathways in plant diterpenoid metabolism *Andrew O'Donnell (GER), p. 10*
11:15	5 - In vivo validation of the *Nepeta* iridoid biosynthesis pathway *Lira Palmer (SOC), p. 11*
11.30	***Lunch break*** *Bring your own lunch*

2nd talk session *chair: Marilia Freire*	
"Olfaction and taste in insects"	
12:30	6 - Calmodulin regulates the olfactory performance in *Drosophila melanogaster* *Kalpana Jain (HAN), p. 12*
12:45	7 - Performance differences of olfactory receptors *Lorena Halty (HAN), p. 13*
13:00	8 - The hunt for identifying sex pheromone genes in the moth *Heliothis subflexa* *Elise Fruitet (Groot), p. 14*
13:15	9 - Deorphanization of gustatory neurons in *Drosophila melanogaster* *Venkatesh Pal Mahadevan (HAN), p. 15*
13:30	***End***

Wednesday, July 01st, 2020

08:50	**Arrival:** *Join Zoom Meeting (register first!)* *https://zoom.us/j/95019661394?pwd=NGlEb2tqYjJrQnBBYkg2SHgzUVRpQT09*
3rd talk session *chair: Claudia Voelckel*	
"Microbial communities in different contexts"	
9:00	10 - Phylogenetic and metabolic dissimilarity between interacting genotypes facilitates cross-feeding in bacteria *Samir Giri (Kost), p. 16*
9:15	11 - A closer look into the guts of *Spodoptera littoralis* larvae: a microbial perspective *Tilottama Mazumdar (BOL), p. 17*
9:30	12 - Genome-wide characterization of rhizobia nodulating with common beans in western Kenya *Clabe Wekesa (OelmüllerL), p. 18*
9:45	13 - Modelling population dynamics in a *Bacillus subtilis* biofilm using a minimal model and game theory *Ravindra Garde (Schuster), p. 19*
10:00	14 - Identification of new compatible solutes in marine microalgae *Simona Fenzia (Pohnert), p. 20*
10:15	***Coffee break(out)s:*** 1 - PhD rep meet & greet (Emily Puckett) 2 - Introducing the MPI-CE podcast channel: Synthetic Opinion (Sin Prelic) 3 - PhD almost done - what's next? (Karin Groten) 4 – Publishing and thesis issues (Kirsten Heinrich, Angela Günter) 5 - How do I market my research and fulfill my PR requirements? (Angela Overmeyer) 6 – IMPRS faculty meeting (Jonathan Gershenzon) 7 – Just coffee and chat (for non-PhDs and non-IMPRS faculty) 8 – Guest speaker Q&A (Silke Allmann)

	4th talk session *chair: Sin Prelic*
"Chemistry of insect defenses and plant carnivory"	
10:45	15 - Venom deployment, composition and function in predatory Heteroptera *Maike Fischer (HEC), p. 21*
11:00	16 - Profiling the defensive secretion of the large milkweed bug (*Oncopeltus fasciatus*) raised on different diets *Paola Rubiano-Buitrago (ROW), p. 22*
11:15	17 - *Nepenthes x ventrata* extrafloral nectar chemistry is tailored for prey attraction *Alberto Davila-Lara (MIT), p. 23*
11:30	***Lunch break*** *Bring your own lunch*
12:45	All jury members submit their scores and rankings
13:30	**Results of IMPRS course survey** *Claudia Voelckel*
13:45	**Announcement of talk awards** *Jonathan Gershenzon*

Coffee break(out)s:

1 - PhD rep meet-and-greet (Emily Puckett)
The MPI-CE PhD representatives: who we are, what we do, and why we are

2 - Introducing the MPI-CE podcast channel: Synthetic Opinion (Sin Prelic)
An in-house life science podcast that grapples with demystifying the life of a daily scientist and their work. A channel with two formats: expert 1-on-1 interviews, and conversational round table discussions about the testimonials, trials and tribulations of a research life in science.

3 - PhD almost done - what's next? (Karin Groten)
Ask Karin about PostDoc funding.

4 - Publishing and thesis issues
How to:

- *keep or share your rights: Copyright and open licenses*
- *provide research data open and safe*
- *my publication Open access?*
- *public author IDs*

5 - How do I market my research and fullfil my PR requirements? (Angela Overmeyer)
Ask Angela about promoting you and your research to the public - online and offline.

6 – IMPRS faculty meeting (Jonathan Gershenzon)
An update for IMPRS faculty members

7 – Just coffee and chat (for non-PhDs and non-IMPRS faculty)

8 – Guest speaker Q&A (Silke Allmann)
Silke answers question on her science and on becoming a group leader.

Talks

Plenary Talk

Scent and scentability: Understanding specificity in plant volatile signaling

Silke Allmann [1]

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Plants under attack are able to emit large amounts of volatiles in the air. These volatiles can profoundly change the behavior of insects interacting with the plant, but they also affect the metabolism of the emitter itself and of those plants growing in close vicinity to the emitter. Our research focuses on a group of plant volatiles earliest emitted upon herbivory, called “green leaf volatiles” (GLVs). We uncovered enzymes present in plants and insects profoundly affecting multiple ecological interactions by converting the highly abundant GLV *Z*-3-hexenal into *E*-2-hexenal. These two compounds, as well as their derivatives, have distinct effects on the behavior of herbivorous and predacious insects as well as on the metabolism of plants. In this talk I will provide an overview of some recent insides in green leaf volatile production and metabolism, discuss the importance of specificity for volatile signaling and speculate about the mechanism of volatile perception in plants

Talk 1

Volatile emission from endophytic fungi colonizing black poplar leaves

Christin Uhe[1], Tobias Köllner[1], Pamela Baumann[2], Beate Rothe[1], Katrin Luck[1], Peter H.W. Biedermann[2], Sybille B. Unsicker[1]

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Plant volatiles play a major role in plant-insect interactions, as defense compounds or attractants for insect herbivores. Endophytic fungi isolated from plants were also found to produce volatiles and this raises the question whether and how these fungal volatiles influence plant-insect interactions. In this study we qualitatively investigated the volatiles released from 15 endophytic fungal species isolated from leaves of old-growth black poplar (Populus nigra) trees. Fungal volatiles were collected with polydimethylsiloxane (PDMS) tubes and analyzed by GC-MS coupled to a thermodesorption unit. The volatile blends of these endophytes comprise typical fungal compounds, as well as components characteristic of the constitutive and herbivore-induced volatile blend of poplar trees. For one endophytic species, we characterized two sesquiterpene synthases that produce volatiles also known from black poplar trees. A number of fungus-derived volatiles like 2-phenylethanol, isoamyl alcohol or some sesquiterpenes e.g. (E)-β-caryophellene are already known to play a role in direct and indirect plant defense. Thus, we argue that the emission of volatiles from endophytic microbial species should be considered in future studies investigating tree-insect interactions.

Talk 2

Arabidopsis response to a S-containing fungal volatile from *M. hyalina* under sulfur deficiency

Yu-Heng Tseng[1], Ralf Oelmüller[1]

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Sulfur is an important macronutrient required for plant growth. Sulfur deficiency induces the expression of sulfate transporter genes, and increases breakdown of sulfur-containing metabolites for primary growth. We identified a sulfur-containing volatile produced by the plant-growth-promoting beneficial fungus Mortierella hyalina. Co-cultivation of the fungus with Arabidopsis seedlings or application of low dosages of the pure volatile promote growth under sulfur deficient conditions. The fungal volatile represses the expression of sulfate transporter genes as well as the sulfur deficiency marker gene SDI1 (SULFUR DEFICIENCY-INDUCED1) under sulfur deficiency, the latter gene codes for an enzyme responsible for glucosinolate catabolism. Correspondingly, application of the fungal volatile increased the glucosinolate and glutathione contents even under sulfur deficiency. Moreover, application of the volatile to the slim1 mutant, which cannot respond to sulfur deficiency properly, restored the wild-type phenotype. To understand how the volatile is incorporated into plant body, biochemical assays on cysteine biosynthesis will be carried out. Labeling sulfur atoms in the fungal volatile will also be performed to understand where they end up in the plant. Taken together, this study investigates the beneficial effect of the sulfur-containing volatile from fungus M. hyaliana on Arabidopsis growth under sulfur limitation condition, emphasizing on how the fungal volatile can be incorporated into plant body and be used as sulfur source under sulfur-limiting conditions.

Talk 3

Aggregation of β-Glucosidases in herbivore-damaged maize

Diana Radisch[1], Matthias Erb[2], Jonathan Gershenzon[1], Tobias G. Köllner[1]

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[2]Institute of Plant Sciences, University Bern, Bern, Switzerland

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β-Glucosidases (β-D-glucopyranoside glucohydrolases, E.C. 3.2.1.21) are found in all domains of living organisms, where they play essential roles in the removal of nonreducing terminal glucosyl residues from saccharides and glycosides. Plant β-glucosidases are involved in many physiological processes like cell wall formation, phytohormone release and plant defense. Activation of secondary metabolites by de-glycosylation is a widespread anti-herbivore defense strategy that allows plants to store harmless glycosides and hydrolyze them to toxic products upon attack. The main insect resistance factors in maize are 1,4-benzoxazin-3-one derivates (Benzoxazinoids), like DIMBOA-Glc and DIM2BOA-Glc, which are stored as glucosides and activated by plant β-glucosidases. In maize, two β-glucosidases (ZmGlu1 & ZmGlu2) have been reported to hydrolyze DIMBOA-Glc (2-O-β-d-glucopyranosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one). A sequence analysis showed that ZmGlu1 and ZmGlu2 form a small gene subfamily with six additional ZmGlu genes (ZmGlu3-8). To gain insights into the specific biochemical activity of ZmGlu3-8, the genes were cloned, heterologously expressed in Escherichia coli, and assayed in vitro with artificial substrates and single benzoxazinoids. A kinetic characterization revealed different substrate specificities for individual ZmGlu enzymes.

Remarkably, some maize lines possess only marginal soluble β-glucosidase activity after tissue disruption. In these lines, β-glucosidases are bound to specific proteins called β-glucosidase aggregating factors (BGAFs) and form insoluble but active protein complexes. BGAF-β-glucosidase aggregation has been suggested to protect β-glucosidases from proteolytic degradation in the insect gut. However, the biological function of maize BGAFs, even in the context of β-glucosidase stabilization, is yet unclear. In contrast to the β-glucosidase genes, which showed organ-specific expression patterns not influenced by herbivory, the identified BGAF genes were equally expressed throughout the plant and induced upon herbivory. Potential interactions of purified recombinant BGAF proteins with recombinant ZmGlu proteins were tested with gel shift assays in vitro.

Altogether, our data suggest a potential role of β-glucosidase-aggregating factors combined with specialized β-glucosidases in defense against insect herbivores in maize.

Talk 4

Evolution of Enzyme Pathways in Plant Diterpenoid Metabolism

Andrew J O'Donnell[1,2], Yoko Nakamura[1,3], Reuben J Peters[4], Jonathan Gershenzon[1,2], Axel Schmidt[1,2]

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Diterpene resin acids are C20 tricyclic terpenoids containing a carboxylic acid group that are abundant in conifers. These secondary metabolites require the cyclization of geranylgeranyl diphosphate (a 20-carbon phosphorylated isoprene) by diterpene synthases (diTPS; Geisler et al. 2016) and further oxidation by cytochrome P450 monooxygenases (CYP450). The evolution of diterpene resin acids in the conifers provides an opportunity to test hypotheses about the patchwork hypothesis because the specific actions of both diTPS and CYP450 in conifers appear to be an evolutionary novelty in gymnosperms. CYP450 enzymes implicated in the biosynthesis of diterpene resin acids, termed CYP720B (Geisler et al. 2016), duplicated multiple times in conifers. Conversely, the gene sequence of its angiosperm orthologue (CYP720A) remained as a single genomic copy in all lineages studied. Interestingly, diTPS enzymes that provide substrates to CYP720B enzymes also duplicated after conifers and angiosperms diverged, indicating that diTPS and CYP720B gene families were recruited together for resin acid biosynthesis sometime after the two lineages diverged. This study combines biochemical and phylogenetic analysis of modern-day CYP720B enzymes, and examines the enzymatic activity of the angiosperm CYP720A, to better understand the ancestral conditions that preceded the neofunctionalization apparent in the CYP720B enzymes. Intriguingly, experiments that manipulated CYP720A expression levels in Populus canescens reveal a potential role for CYP720A in defense signaling, in contrast to the roles of orthologous genes found in the gymnosperms. Additional roles for the angiosperm CYP720A in defense signaling are also explored at the molecular level.

Talk 5

In Vivo Validation of the Nepeta Iridoid Biosynthesis Pathway

Lira Palmer[1,4], Ling Chuang[2], Benjamin Lichman[3], Omar Kamileen[1], Nestor Hernandez[1], Sarah O'Connor[1], Mint Consortium[5]

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[2]Institut für Botanik, Leibniz Universität Hannover, Germany
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The seco-loganic pathway is a well-studied plant specialized biochemical pathway responsible for creating ~3000 monoterpenoid indole alkaloids, many used in medicine. The early iridoid pathway creates the initial iridoid scaffold, nepetalactol, a compound of economic interest itself. The Nepetoideae sub-family of the Lamiaceae has lost a key enzyme in the early pathway, iridoid synthase (ISY). However, members of the Nepeta genus, including catnip (Nepeta cataria) and catmint (Nepeta mussinii), can produce nepetalactone iridoids. Previously, members of the O'Connor lab identified and characterized the in vitro activity of the key enzymes in the iridoid biosynthesis pathway, including a convergently evolved ISY. Curiously, a novel set of enzymes, the nepetalactol-related short-chain-dehydrogenase enzymes (NEPS), have evolved within the Nepeta genus and have been identified to control the production of different nepetalactone isomers. In our work, it has become clear that production of these isomers varies amongst species and even individual varieties. This pathway has not been validated in vivo, and mechanisms on carbon flux regulation between isomers are not understood. I adapted the virus-induced gene silencing (VIGS) tool to study this pathway in Nepeta. By targeting key genes of the pathway for expression knockdown, I have validated these genes in vivo and aim to untangle the mechanisms behind isomer regulation in nepetalactone production.

Talk 6

Calmodulin regulates the olfactory performance in *Drosophila melanogaster*

Kalpana Jain[1], Sofia Lavista-Llanos[1], Bill S Hansson[1*], Dieter Wicher[1*]

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Odorant receptors (ORs) are essential for the capability of insects to detect volatile chemical cues with high sensitivity. ORs operate as ligand-gated non-selective cation channels and are formed by heptahelical OrX and Orco (co-receptor) proteins. A highly conserved calmodulin (CaM) binding site (CBS) 336SAIKYWVER344 within the second intracellular loop of Drosophila melanogaster Orco has been shown to be a target for regulating OR performance. In the present study, we asked how a point mutation in the CBS in the Orco protein may affect the olfactory performance of Drosophila melanogaster. We first asked how the point mutation K339N in the Orco CBS would affect the odor responses in Drosophila olfactory sensory neurons (OSNs). Using Ca2+ imaging in an ex-vivo antenna preparation, we first elicited odor responses using the synthetic Orco agonist VUAA1 in all Orco expressing neurons (OrX/Orco). In a next attempt, we restricted the OR spectrum to Or22a expressing neurons (Or22a/Orco) and stimulated these OSNs with their ligand ethyl hexanoate. In both approaches, we found that Drosophila melanogaster flies carrying the K339N point mutation in Orco CBS display a reduced olfactory response. We also found that the K339N point mutation abolishes OSN sensitization. Finally, we asked whether K339N might affect the odor localization performance. By means of a wind tunnel bioassay, we found that odor localization in mutant flies was severely affected by K339N point mutation. In this study, we show that the CBS in the Orco protein plays an important role in shaping the odor response of OSNs, in sensitizing OSNs by repeated weak odor stimuli, and for the odor localization performance of the fly.

Talk 7

Performance differences of olfactory receptors

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Odorant receptors (ORs) are a special class of olfactory receptors that help insects navigate through a complex odor environment. ORs form ligand-gated ion channels composed of a specific ligand-binding subunit (OrX) and a highly conserved co-receptor protein (Orco) located in the dendrites of olfactory sensory neurons (OSNs) and maxillary palps. An interesting property of certain ORs is that they can be sensitized, meaning that near-threshold odorant stimulation can elicit a response in OSNs when the stimulus is repeated within a specific time window.

However, it remains elusive whether it is a general property of all ORs.

In the present study we investigate the differences in performance of two different odorant receptors, the broadly tuned Or22a receptor against the narrowly tuned Or56a receptor, after near-threshold odorant stimulation.

We do so by means of calcium imaging techniques and pharmacology in an antenna ex-vivo preparation of the fruit fly Drosophila melanogaster.

Talk 8

The hunt for identifying sex pheromone genes in the moth *Heliothis subflexa*

Elise Fruitet[1,2], Emily Burdfield-Steel[2], David G Heckel[1], Astrid T Groot[1,2]

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Sexual signals are key components in mate finding and mate choice and their evolution are 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 and ratios of the different components. Even though acetate esters are the most common sex pheromone compounds in Lepidoptera, no genes underlying their production have been identified yet. Our aim is to identify which gene(s) are responsible for the production of acetate esters.

Previous genetic analyses have identified two major QTLs underlying acetate production in *Heliothis subflexa*. Moreover, RNAseq analyses highlight four candidate genes on one of these QTLs. In this study we successfully knocked out two of these candidate genes using CRISPR/Cas9, which led to a change in the acetate ratio of the sex-pheromone. We also identified one extra candidate gene present on the other QTL, which we expected to be reducing acetates when functional. However, while we did find that when this gene was not functional due to the presence of a transposable element insert, acetate production was altered, deactivation of the gene actually led to reduced acetate production. In order to confirm this finding, we also knocked out the gene using CRISPR/Cas9. Our results highlight the complexities of sex-pheromone production and could be used to improve the synthesis of lures for pest-control management.

Talk 9

Deorphanization of gustatory neurons in *Drosophila melanogaster*

P.M.Venkatesh[1], Sofia Lavista Llanos[1], Bill Hansson[1] and Markus Knaden[1]

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Chemosensation is essential for the survival of insects. Activities like searching for food, mating, and oviposition in the fruit fly, *Drosophila melanogaster* are at least partly governed by chemical cues detected via olfaction and gustation. This chemical information is conveyed to the higher brain centers via diverse olfactory sensory neurons (OSNs) and gustatory sensory neurons (GSNs) expressing olfactory receptors (ORs) and gustatory receptors (GRs) respectively. ORs are exclusively expressed on the antenna and on maxillary palps while GRs are predominantly expressed on labellum, tarsi, genetalia etc. We aim to deorphanize some of those GRs in *D. melanogaster* that are conspicuously expressed on the antenna, and try to identify their contribution to the fly's behavioural output. Initially, we tested expression of 16 GRs using the Gal4-UAS binary expression system and observed that 9 GRs are expressed on the antenna. Further, from these 9 GRs, we aim to characterize the function of three gustatory receptors (Gr64b, Gr64e and Gr93a) using various techniques such as electrophysiology, immunofluorescence, calcium imaging as well as various behavioural assays. Lastly, with this project, we wish to increase our understanding of the fly's chemosensation by identifying natural ligands for orphan neurons and their role in the overall behaviour.

Talk 10

Phylogenetic and metabolic dissimilarity between interacting genotypes facilitates cross-feeding in bacteria

Samir Giri[1,2], Leonardo Oña[2], Silvio Waschina[3], Shraddha Shitut[1,2], Ghada Yousif[1,2], Christian Kost[1,2]

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Metabolic cross-feeding, wherein one microorganism consumes metabolites provided by another, is a ubiquitous feature of microbial communities. Empirical studies have shown several benefits of cross-feeding such as, (i) promoting diversity in nutrient-poor environments, (ii) degradation of toxic compounds and, (iii) increased growth. By exchanging metabolites, bacteria can save metabolite biosynthesis cost and hence enhance consortium-level growth. However, it is unclear how relatedness between genotypes affects the outcome/interaction within a microbial community. In order to test whether two more closely related genotypes are likely to engage in a cross-feeding interaction than two distantly related strains, I combine different wild-type (Donor) strains with auxotrophs (recipient) of varying phylogenetic distances, and ask how cross-feeding between the donor-recipient pair is affected. Our work finds that all donors support the growth of auxotrophic recipient in cocultures. Determining growth across pairs of donor-recipient strains, we find a strong correlation of recipient growth with increasing phylogenetic and metabolic distances. Moreover, our result demonstrates that a diverse amino acid pool produced by the donor drives this cross-feeding. Interestingly, analyzing the cost of growth support in donors revealed the emergence of bidirectional cross-feeding from uni-directional byproduct interactions. The results from this study provide unique insights into the rules that govern cross-feeding within microbial communities and may thus help to explain the widespread distribution of such interactions.

Talk 11

A closer look into the guts of *Spodoptera littoralis* larvae: a microbial perspective

Tilottama Mazumdar[1], Beng Soon Teh[1], Aishwariya Murali[1, 2], Wilhelm Boland[1]

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The complex interaction amongst a higher organism and its resident gut flora is a subject of immense interest in the field of symbiosis. Insects harbor a population of gut bacteria that play roles in their growth, development and immunity. There exists a variation in the microbial population with the development of the insect.
The gut microbiota of Spodoptera littoralis, a Lepidopteran pest, varies spatially and temporally. The core community consists of Enterococci, Lactobacilli and Clostridia. The selection of one bacterial species over the other is quite evident throughout the lifecycle, so is the differing bacterial population and abundance among the fore, mid and hind gut of the larva. By the time the larva reaches fifth instar, Enterococcus mundtii persist and dominate. The gut environment dictates the persistence of its residents. There is a pH gradient from alkaline to neutral along fore to hind gut respectively, and a depleted iron condition as posed by the chelator 8-HQA (acid) produced by the insects.
We ask the following: **How does the E. mundtii dominate by surviving the gut stress? What kind of interaction goes on between them and their host? Finally, how does 8-HQA define the microbial landscape of the S. littoralis gut?**
A GFP-tagged reporter E. mundtii has been constructed to answer the **first two questions**. They are fed to the insects at early instars, and sorted from the gut spatially and temporally using flow-cytometry. These reporter bacteria which had integrated into the gut community of the larva must have changed their gene expression profile according to the new environment. A transcriptomic analysis of the retrieved bacteria from the host gut should answer our questions. The fluorescent reporter confirmed the persistence of E. mundtii in the gut. Also, RNA-sequencing of the sorted bacteria has informed us about various strategies of the symbiont's survival. There are upregulated pathways for stress tolerance: alkaline stress, biofilm formation, two-component signaling systems, resistance towards oxidative stress. There is a differential regulation among various metabolic pathways too. Although these symbionts seem to depend on the host for amino acid and fatty acids, they have an enriched lysine synthesis pathway in the hindgut of the larva, indicating their lysine contribution (an essential amino acid) towards the host.
To address the **third question**, a knockout line of larvae for 8-HQA production was constructed using the CRISPR-Cas9 method, whose gut microbiota was elucidated and their differences with the wild type strains were analyzed. The results give us an idea of the importance of host-genetics in dictating the microbial composition and numbers in the guts of hosts.

Talk 12

Genome-wide Characterization of Rhizobia Nodulating with Common Beans in Western Kenya

Clabe Wekesa[1], Ralf Oelmuller[1], Alexandra C.U. Furch[1]
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Common bean is not only a source of essential nutrients but also a medicinal plant. It is a source of proteins, carbohydrates, vitamin B complex and essential minerals for human nutrition. Flavonoids and isoflavonoids, mainly produced as defense compounds against phytopathogens, and antioxidants, act as anti-cancer agents. Its production is restricted by the poor soil fertility, in particular, limitations in nitrogen. Fertilizer application is expensive and pose environmental challenges. Soil inoculation with rhizobia is an inexpensive alternative to improve nutrient enrichment through nitrogen fixation. Foreign inoculants that have been applied in Kenyan soils over time have failed to maximize the production due to challenges associated with adaptability in Kenyan soils. Indigenous rhizobia colonizing common beans in Kenyan soils and their symbiotic ability remains largely unknown and therefore inoculant development specifically for Kenyan soils has been delayed. This study was therefore performed to isolate, test for symbiotic efficiency and characterize indigenous rhizobia nodulating common beans in Western Kenya soils. Among the ten rhizobia isolates, three strains were found to be symbiotically efficient and therefore their genomes were sequenced for characterization. Circular representation of the genomes were 6,908,572 bp, 6,778,822 bp and 10,260,409 bp for isolates BGM003, BUS002 and BUS003 respectively. Isolates BGM003, BUS002 and BUS003 had 6747, 6851 and 10207 genes respectively which were classified into 26 subsystems representing various biological processes. Isolate BGM003 and BUS002 had a close phylogenetic relationship with *Rhizobium phaseoli* with DDH values of 89.2% and 95.9% respectively. However, though *Rhizobium phaseoli* had the closest phylogenetic relationship with BUS003, DDH value was only 60%. All the three isolates shared 68.8% genes among themselves and the reference *Rhizobium phaseoli*. However, 18.2% genes were exclusively for isolate BUS003 as compared to only 2.7% and 1.5% genes exclusive for isolates BGM003 and BUS002. **This evidence can point to a conclusion that isolates BGM003 and BUS002 are strains of *Rhizobium phaseoli* while isolate BUS003 is a novel species that closely relate to *Rhizobium phaseoli*.**

Talk 13

Modelling population dynamics in a Bacillus subtilis biofilm using a minimal model and game theory

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Bacillus subtilis is a widely studied model system and is especially important in the field of social biology due to its ability to form biofilms. It is also known to form spores and elaborate structures called fruiting bodies for the dispersal of these spores. Many efforts have been made to study the population dynamics of this complex community of cells co-existing as a well-coordinated unit. Minimal models are powerful tools that can help us understand complex phenomena and since they are minimal, the analysis of these models is quite easy. Game theory is another powerful tool that can help us understand the costs and benefits of the decision a particular *B. subtilis* cell takes when faced with the challenges of the environment. This work is a binocular view of the population dynamics of a *B. subtilis* biofilm through the objectives of minimal modelling and game theory. This study suggests that even at low costs of biofilm production, we see spikes in the proportion of sporulators indicating the presence of sporulation cycles.

Talk 14

Identification of new compatible solutes in marine microalgae

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Diatoms are photosynthetic unicellular algae, responsible for 20% of global carbon fixation and 40% of marine primary production. They are important producers of zwitterionic metabolites, a class of small organic compounds, classified as “compatible solutes”, with central osmoregulatory, antioxidant and cryoprotectant functions. Dimethylsulfoniopropionate (DMSP) is the main representative of this class of molecules: it is a sulfur-containing metabolite reaching high cellular concentrations in marine algae. Marine bacteria and algae metabolize DMSP into the correspondent volatile compound dimethylsulfide (DMS), contributing to the flux of sulfur from the hydrosphere to the atmosphere. Many organic osmolytes produced by microalgae were identified and their contribution to the osmoadaptation and osmoregulation of marine ecosystems were pointed out, but a complex picture emerges, showing that the common DMSP/DMS concept represents a massive oversimplification. Osmoadaptation depends on a plethora of metabolites of which only few have been structurally elucidated. In this talk, I will present how the development of direct analytical methods in LC\MS analysis allows the detection, identification and quantification of novel zwitterionic metabolites in marine algae. Their regulation under osmotic stress and their ecological functions will be discussed.

Talk 15

Venom deployment, composition and function in predatory Heteroptera

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The Heteroptera are a diverse suborder of phytophagous, hematophagous and zoophagous insects. The shift to zoophagy involved the transformation of salivary glands into so-called venom glands and predatory Heteroptera use their venom not only to immobilize and pre-digest animal prey but also to defend themselves against both vertebrate and invertebrate enemies. With an integrated transcriptomics and proteomics approach, we analyzed the composition of venoms from the anterior main gland (AMG) and posterior main gland (PMG) of several zoophagous Heteroptera. In the African assassin bugs Platymeris biguttatus L. and Psytalla horrida Stål, the AMG and PMG secreted distinct, complex protein mixtures with few interspecific differences. Furthermore, our study revealed remarkable differences in the biological activity of AMG and PMG venom in both species and a context-dependent use of the two venom types in P. horrida but not in P. biguttatus. In accordance with the African species, we also found distinct protein compositions of AMG and PMG venom in several European Heteroptera. However, none of these species possessed a context-dependent deployment of the two venom types. Instead, all species used PMG venom for prey overwhelming, digestion and defense, thus leaving the role of the AMG unclear. To further characterize the venom of selected species, we will fractionate the protein mixtures using FPLC and assay the biological activity of the fractions. This will help to resolve overall venom function as well as effects of individual venom components. The results of our studies contribute to a better understanding of the ecology of predatory Heteroptera and the identification of venom components with in vivo activities potentially relevant for medical applications.

Talk 16

Profiling the defensive secretion of the large milkweed bug (*Oncopeltus fasciatus*) raised on different diets

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The large milkweed bug (Oncopeltus fasciatus) is a specialized migratory insect that feeds on milkweed seeds (Asclepias spp.), although it can survive on various natural and artificial seed-based diets under laboratory conditions. Plants of the genus Asclepias produce cardenolides: steroid toxins that interfere with Na+/ K+ ATPase function in most herbivores. O. fasciatus is adapted to feed on Asclepias through mutated Na + / K + ATPases that confer reduced sensitivity to cardenolides. They sequester these compounds and release them as part of a defensive secretion that deters predators. Previous studies focused on the total content of cardenolides in adult secretion, selectivity and excretion of cardenolides using model substances or selected Asclepias seeds with different metabolic profiles in thin layer chromatography. To our knowledge, there are no studies on cardenolide secretions in the nymph stage, but they are also considered unpalatable for predators.

From the information above, we focus our research on the chemistry behind these phenomena, starting on which compounds are released into the defense secretion of Oncopeltus fasciatus and the variability of the chemical profile of this substance according to the insect diet. Given the fact that these insects use a variety of milkweed in their migration pattern across America, we compare the secretion of individuals of O. fasciatus when raised on non-toxic Helianthus annuus (sunflower seeds), Asclepias curassavica and A. incarnata. We analyze the secretion of O. fasciatus using HPLC-MS and NMR experiments, in both nymph and adult stages (because nymphs and adults are known to store and release secretion differently). We made this comparison together with a phytochemical study of Asclepias seeds, to obtain reliable data on the metabolites present in the diet. Results have shown the relevance of triacylglycerides as the mayor component of the secretion, along with a metabolite that seems to appear regardless of diet variation. The presence of cardenolides in the defensive secretion on O. fasciatus raised on Asclepias is being confirmed in both nymphs and adults, yet there are differences in the profiles between life stages, and some compounds hint the possibility of chemical transformation during the sequestration process.

Talk 17

Nepenthes x ventrata extrafloral nectar chemistry is tailored for prey attraction

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Carnivorous plants often live on poor soil and hunt for insect prey in order to get additional nutrients such as nitrogen and phosphate. Nepenthes x ventrata (Nv), a carnivorous plant from the Philippines, developed a pitcher-trap where prey killing and degradation occurs due to the presence of a digestive pitcher fluid. Nutrients from digested prey, mainly ants, are taken up in the pitchers. Some plants offer food to ants as reward for indirect plant defense service, often as extrafloral nectar (EFN). Main components of EFN are sugars (fructose, glucose, sucrose) and amino acids (AAs), providing energy and nutrients. In Nepenthes, EFN production is found at leaf branches and on the peristome, the pitcher-trap opening involved in prey attraction and capture. Likely, in Nepenthes ants are not attracted for indirect defense but as prey. Therefore, we studied the role of EFN in N. x ventrata. Combining different approaches, we characterized the chemical composition of NvEFN and compared the results with results from ant-plant species, which suggest different ecological implications for NvEFN. From the plants' perspective, AAs investment in the nectar is counterproductive as Nepenthes is hunting for nitrogen. Hence, the plant invests valuable resources only as a gift at the branches but the closer to the peristome the nutritional quality of the nectar decreases. We also discovered a fluorescent compound produced by EFN and tested the fluorescence as visual cue in ant behavioral experiments. The results will be discussed in the context of plant carnivory and its ecology.