IMPRS

Exploration of Ecological Interactions with Molecular and Chemical Techniques

18th IMPRS Symposium

March 13-14, 2019

Old Castle, Dornburg

Program

Tuesday, March 13th, 2019

08:00	*Departure, central bus stop (opposite Jena Paradies train station)*
08:45	**Welcome and Announcements** (Großer Kaisersaal) *Prof. Jonathan Gershenzon, Dr. Claudia Voelckel*
09:00	**Plenary Lecture 1** (Großer Kaisersaal) How herbivores exploit multifunctional plant metabolites *Prof. Matthias Erb, University of Bern, Switzerland,* p. 9 *Chair: Diana Radisch*
10:00	***Coffee break (Rittersaal)***

	1st talk session (Großer Kaisersaal) *chair: Franziska Eberl*
Part 1 - How plant antagonists imitate and manipulate plant metabolites	
10:30	1 - Plants vs. fungi? – How fungal infection alters auxin levels in *Arabidopsis* roots *Anja Meents (BOL), p. 11*
10:50	2 - *Plutella xylostella* glucosinolate sulfatase - more than a counter-adaptation to the plant mustard oil bomb? *Ruo Sun (GER), p. 12*
11:10	3 - Chlorophyll detoxification? Learning from Spodoptera littoralis *Vincensius Oetama (BOL), p. 13*
11:30	4 - Distribution of plant cell wall degrading enzymes in beetles of the family Cerambycidae *Na Ra Shin (HEC), p. 14*
12:00	***Lunch (Rittersaal)***
13.00	**Poster talks 1** (Großer Kaisersaal) Odd numbers (1 slide and 1 min/poster)
13:15	**Poster session 1** Odd numbers (Rittersaal)
14:45	***Coffee break (Rittersaal) & group photo***

Program

Tuesday, March 13th, 2019

	2nd talk session (Großer Kaisersaal) *chair: Matilda Gikonyo*
Part 2 - Smells like plasticity: learning odors in flies and hawkmoths	
15:15	5 - Evolution of sex pheromones in *Drosophila* *Mohammad Khallaf (HAN), p. 15*
15:35	6 - Experience-dependent plasticity of an aversive olfactory circuit in *Drosophila melanogaster* *Benjamin Fabian (HAN), p. 16*
15:55	7 - Tongue twister: Hawkmoths do not learn odors that they perceive with their proboscis *Elisabeth Adam (HAN), p. 17*
16:15	**PhDNet survey & course offerings** (Großer Kaisersaal) *Wiebke Häger*
	IMPRS Faculty Meeting (Kleiner Kaisersaal) Claudia Voelckel
16:35	Counselling at the MPI-CE (Großer Kaisersaal) *Renate Ellinger*

17:00	Bus returns to Jena
18:00	Dinner with guest speakers – Zur Noll
19:00	Discussions with guest speakers and MPICE scientists about collaborative projects

20:30	End of day 1

Program

Wednesday, March 14th, 2019

08:00	*Departure, central bus stop (opposite Jena Paradies train station)*
09:00	**Plenary Lecture 2** (Großer Kaisersaal) Chemical ecology of pyrrolizidine alkaloids – the evolution of a defensive trait *Prof. Dietrich Ober, Kiel University, Germany, p. 10* *chair: Marie Pauline Sell*
10:00	***Coffee break (Rittersaal)***

	3rd talk session (Großer Kaisersaal) *chair: Chloe Langley*
Part 3 - News from the world of plant defensive chemistry	
10:30	8 – Fungal infestation induces O-methylation of flavonoids in maize *Christiane Förster (GER), p. 18*
10: 50	9 – At the edge: How the macronutrient sulfate is used to modify the secondary metabolites salicin and salirepin in poplar *Nathalie Lackus (GER), p. 19*
11:10	10 – Forisomes - possible key players in legume defense against aphids *Maria Paulmann (GER), p. 20*
11:30	11 – *Novel zwitterionic metabolites from marine diatoms* *Simona Fenizia (FSU), p. 21*
12:00	***Lunch (Rittersaal)***
13.00	**Poster talks 2** (Großer Kaisersaal) Even numbers (1 slide and 1 min/poster)
13:15	**Poster session 2** (Rittersaal) even numbers
14:45	***Coffee break (Rittersaal)***

Program

Wednesday, March 14th, 2019

4th talk session (Großer Kaisersaal) *chair: Elisabeth Adam*	
Selected topics from plant pollination, mycorrhiza and mass spectrometry	
15:15	12 - Uncoupling pre- and post-pollination in *Nicotiana attenuata* to evaluate the potential and actual outcrossing of different pollinators *Julia Bing (ITB)*, p. 22
15:35	13 - Low abundances of irMPK4 plants in population increase total population yield, but only without AMF interactions *Erica McGale (ITB)*, p. 23
15:55	14 - Mass spectrometry imaging on plants - the ups and downs of method development *Benjamin Bartels (MS)*, p. 24
16:15	**Closing remarks** (Großer Kaisersaal) *Prof. Jonathan Gershenzon*
16:25	Talk and poster jury meets to cast votes (Großer Kaisersaal)
16:45	Bus returns to Jena
18:30	Café Immergrün *Prof. Gershenzon announces winners of talk and poster prizes*
19:00	Symposium feedback discussions and farewell of the guest speakers
20:00	End of day 2

Posters

Posters

13. Single-nucleus transcriptomics of olfactory sensory neurons and support cells in the *Drosophila* antenna
    Sinisa Prelic (HAN), p.38

14. Beetle-induced plant response leads to a shift in feeding preference of *Lymantria dispar* caterpillar
    Christin Uhe (GER), p.39

15. Deorphanization of chemosensory neurons in *Drosophila melanogaster*
    Venkatesh Pal Mahadevan (HAN), p.40

16. Nascent secondary metabolites: Evolution of early iridoid synthesis in *Nepeta*
    Lira Palmer (SOC), p.41

17. Towards FDR estimation in computational metabolomics
    Martin Hoffmann (FSU), p.42

18. Terpene emission in a combination of drought stress and methyljasmonate treatment in a conifer species *Picea glauca*
    Erica Perreca (GER), p. 43

19. Differential equation based minimal model describing metabolic oscillations in Bacillus subtilis biofilms
    Ravindra Garde (FSU), p.44

Talks

Plenary 1

How herbivores exploit multifunctional plant metabolites

Matthias Erb[1]

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Small molecular weight organic compounds are common across the galaxy and transcend all known biological interactions. Plants, in particular, have evolved a remarkable capacity to produce diverse sets of so-called specialized metabolites from a few simple, inorganic precursors. Already 1977, Rhoades argued that plant specialized metabolites are likely multifunctional, i.e. that they serve multiple purposes. Multifunctionality may render the production of specialized metabolites more cost effective and may explain their abundance and tight spatiotemporal control in plants. Work over the last decades confirms that specialized metabolites often have a broad range of functions, from growth and development to defense.

If specialized metabolites have different uses for plants, can adapted natural enemies also use them for multiple purposes? In my presentation, I will explore this question by discussing our work on benzoxazinoids, the most abundant specialized metabolites in grasses such as wheat and maize. We find that benzoxazinoids act as direct defenses, defense signaling molecules, microbiome modulators and siderophores. At the same time, the western corn rootworm, a specialist maize pest and important agricultural pest, exploits benzoxazinoids as foraging cues, protective agents and micronutrient providers. Thus, the multifunctionality of plant specialized metabolites is mirrored in the adaptations of a specialist herbivore, resulting in a tightly interlocked metabolism. These findings have implications not only for the evolution of plant specialized metabolism, but also for the control of agricultural pests through plant-based approaches.

Plenary 2

Chemical ecology of pyrrolizidine alkaloids – the evolution of a defensive trait

Dietrich Ober[1]

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Arbuscular mycorrhiza symbioses between most land plants and fungi of the glomeromycota improve plant performance because the fungi deliver mineral nutrients to their hosts. In return, the fungi receive photosynthetically fixed carbon. It has long been thought that the fungi are only fed with carbohydrates but we and other laboratories have recently discovered that plants serve their symbionts also with lipids and that fungal development and growth depends on this lipid source. Root colonization by arbuscular mycorrhiza fungi involves distinct developmental steps that are largely under plant control and can be genetically separated by plant mutants. These steps include fascinating plant cell rearrangements that precede differentiation of fungal hyphae into particular shapes inside these plant cells. In my research group, we use a combination of genetic, physiological, biochemical and cell biological approaches to uncover and understand the plant molecular mechanisms, which regulate and execute these plant cell rearrangements for fungal accommodation. My presentation will focus on our recent progress in understanding the role of plant hormone signaling and transcriptional regulation in arbuscular mycorrhiza development and function.

Talk 1

Plants *vs* Fungi? – How fungal infection alters auxin levels in Arabidopsis roots

Anja K. Meents[1,2], Alexandra C. U. Furch[2], Marília Almeida-Trapp[1], Sedef Özyürek[2], Sandra S. Scholz[2], Alexander Kirbis[3], Teresa Lenser[3], Günter Theißen[3], Veit Grabe, Bill Hansson[4], Axel Mithöfer[1], and Ralf Oelmüller[2*]

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Auxin (indole-3-acetic acid, IAA) is an important phytohormone among others involved in root growth and development. Root-interacting beneficial and pathogenic fungi utilize auxin and its target genes to manipulate the performance of their hosts for their own needs. In order to study, follow and visualize auxin effects in fungi-colonized Arabidopsis roots, we used the dual auxin reporter construct *DR5*::*EGFP-DR5v2*::*tdTomato* and fluorescence microscopy as well as LC-MS-based phytohormone analyses. We demonstrate that the beneficial endophytic fungi *Piriformospora indica* and *Mortierella hyalina* produce and accumulate IAA in their mycelia, in contrast to the phytopathogenic biotrophic fungus *Verticillium dahliae* and the necrotrophic fungus *Alternaria brassicicola*. When exposed to either of the pathogenic fungi, in Arabidopsis roots the signals of the auxin-responsive reporter genes disappeared within 3 hours. When exposed to *P. indica,* within 1 day significantly higher auxin levels and stimulated expression of auxin-responsive reporter genes were detected in lateral root primordia and the root elongation zone. Elevated auxin levels were also present in the *M. hyalina*/Arabidopsis root interaction, but no downstream effects on auxin-responsive reporter genes were observed. However, here the jasmonate level was strongly increased. We propose that the lack of stimulated root growth upon infection with *M. hyalina* is not caused by absence of auxin, but an inhibitory effect initiated by high jasmonate content.

Talk 2

Plutella xylostella glucosinolate sulfatase, more than a counter-adaptation to the plant mustard oil bomb?

Ruo Sun[1], Jonathan Gershenzon[1], Sagar Pandit [2], and Daniel Giddings Vassão[1]

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The glucosinolate (GLS)-myrosinase system, often called “the mustard oil bomb”, is the characteristic two-component chemical defense system of Brassicaceae plants against herbivores. GLSs and myrosinases are stored in separate compartments to avoid self-intoxication. Chewing by a herbivore ruptures this compartmentalization, and myrosinases hydrolyze GLSs to produce toxic isothiocyanates (ITCs). In the crucifer-specialized herbivore *Plutella xylostella* (diamondback moth, Lepidoptera: Plutellidae), glucosinolate sulfatases (GSS) function as a counter-adaptation to GLSs, efficiently desulfating GLSs to form harmless desulfo-GLSs and preventing ITC formation. To outcompete myrosinases, GSS must be produced n high amounts, requiring substantial resource investment. Here, to explore the influence of GSS on *P. xylostella* physiology and on its multi-trophic interactions, we used plant-mediated RNAi to manipulate its function *in vivo*. Successful *gss* silencing in *P. xylostella* larvae significantly lowered GSS activity and concomitantly elevated the concentrations of toxic ITCs in larval tissues, leading to reduced larval performance. In the next trophic level, the presence of GLS-derived ITCs in *P. xylostella* larvae after gss silencing also strongly affected the performance of its natural enemies. Thus, as an additional consequence of serving as a GLS counter-adaptation, *P. xylostella* GSS also blocks the bottom-up cascading effects of the mustard oil bomb onto higher trophic levels.

Talk 3

Chlorophyll detoxification? Learning from *Spodoptera littoralis*

Vincensius Oetama[1], Stefan Pentzold[1], Yannick Pauchet[1], Wilhelm Boland[1]

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Chlorophyll is the green pigment which can be found ubiquitously in plants, algae, and bacteria. It is classified as a natural product that is needed in the photosynthesis. The degradation pathway and enzymes involved in chlorophyll degradation are known in plants during leaf senescence and fruit ripening. However, chlorophyll degradation mechanisms in chewing insects are not known. The previous study has shown the detected chlorophyll catabolites were similar as in the plants. Moreover, the catabolites are present already in the foregut. In the present study, the suspected liable protein – Chlorophyllide binding protein (CHBP) – has been identified using transcriptome and proteome analysis from regurgitate of *S. littoralis*. The gene functional analysis using RNAinterference has shown the importance of the gene and indicating a metabolic change detected in the feces. We also found a lower survival rate in larvae injected with gene specific dsRNA, where gene expression was decreased up to 80%. Heterologous expressed CHBP in insect cells was used for ligand assay and revealed that not only chlorophyllide, but also chlorophyll could be bind to CHBP. Photo toxicity assay in the expressed and non-expressed CHBP insect cells showed the higher susceptibility in the expressed CHBP insect cells. These findings lead to the understanding this mechanism as a chlorophyll detoxification. Moreover, the alteration of gut's condition compare to feces' condition yielded new finding of several catabolic compounds. Putting together pieces of the puzzle hopefully will bring the whole understanding of the chlorophyll degradation mechanism in Lepidopteran insects.

Talk 4

Distribution of plant cell wall degrading enzymes in beetles of the family Cerambycidae

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Cerambycid beetles represent the most diverse group of xylophagous (wood feeding insects). Larvae of these beetles live in a challenging environment. For their developing, larvae consume woody tissues containing plant cell wall. These structures are composed of recalcitrant polysaccharide and decomposition such as cellulose, hemicellulose and pectin. Previous studies have shown that beetles of the superfamilies Chrysomeloidea (which includes the Cerambycidae) and Curculionoidea have abilities to produce plant cell wall degrading enzymes (PCWDEs) to break down cellulose, hemicellulose and pectin. However, little is known about characteristics and molecular evolution of PCWDEs in cerambycid beetles. Therefore, to expand our knowledge on the evolutionary history and functional characteristics of PCWDEs in Cerambycidae, we sequenced, using RNA-seq, midgut transcriptomes of 22 species representing six out of the eight subfamilies of this family of beetles. Our sequencing effort provides the most extensive genomic/transcriptomic dataset for this group of insects to date and will lead to novel insights into the biology of the Cerambycidae. We screened all putative carbohydrate-active enzymes (CAZymes) present in these 22 transcriptomes to understand their distribution in these beetles. Among them we have identified GH5 subfamily 2 genes in these 22 species and 2 cerambycid beetles' genomes acquired from NCBI database. Through curating PCWDEs encoding genes and phylogenetic analyses, we can lead to new molecular view of points on contribution and distribution of PCWDEs in cerambycid beetles.

Talk 5

Evolution of sex pheromones in Drosophila

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Pheromone receptors represent a gateway between the chemical stimuli and the resultant sexual behaviors. Despite the profound knowledge of *Drosophila melanogaster* sex pheromones, little is known how other drosophilids regulate their social and sexual behaviors. Here we analyze the chemical profiles of 91 species within the genus *Drosophila* to elucidate how species-specific signals can contribute to form premating isolation barriers. chemometric and genetic analyses indicate a phylogenetic correlation to the evolution of the drosophilids' chemical profiles. Through a series of chemical synthesis, we identify previously anonymous male-specific compounds, many of them are transferred to females during copulation. From single molecules and genes, to neurons, to behavioral responses, we dissect the evolution of sex pheromones perception in closely related species living under different ecological conditions. Our results increase the understanding of the evolution of *Drosophila* pheromones and how sexual isolation barriers between species are created mainly by species-specific signals.

Talk 6

Experience-dependent plasticity of an aversive olfactory circuit in *Drosophila melanogaster*

Benjamin Fabian[1], Veit Grabe[1], Rolf G. Beutel[2], Bill S. Hansson[1] & Silke Sachse[1]

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The sense of olfaction is very crucial for insects in order to navigate in a complex environment of volatile odorants. Olfactory cues play a major role in locating suitable substrates for feeding and oviposition and are necessary for finding potential mating partners or for the avoidance of predators and parasitoids. While the structure and function of the olfactory system of *Drosophila melanogaster* is well understood and documented, little is known about whether and to which extent individual experience is able to modify certain parts of this system. In our study we focus on the aversive olfactory circuit that is activated by geosmin, an ecologically highly relevant odorant of toxic mold. Flies are cyclically exposed to geosmin over the duration of four days and afterwards their brains are analyzed via two-photon imaging techniques. In the presentation we show which parts of the olfactory circuit are able to undergo plastic changes and which parts seem to be hardwired.

Talk 7

Tongue twister: Hawkmoths do not learn odors that they perceive with their proboscis

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The hawkmoth *Manduca sexta* is well known for locating flowers and learning floral odors by using its antennae. However, a recent study showed that the moth is also able to smell the advertisement and locate the nectar of individual flowers using the tip of the proboscis. Anatomical as well as electrophysiological analyses showed that multiporous sensilla at the tip of the moth's tongue are able to sense floral compounds.

Talk 8

Fungal infestation induces O-methylation of flavonoids in maize

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O-methylation of plant specialized metabolites is an important mechanism that creates structural diversity and provides compounds with modified chemical properties, helping the plant to cope with their environment and encounter different biotic and abiotic threats. In previous work we have identified and characterized four *O*-methyltransferases (OMTs) (BX10/11/12/14) that are involved in the herbivore-induced metabolism of benzoxazinoid defense compounds in maize. All four enzymes catalyze the same reaction and generate an *O*-methylated product that is even more toxic to herbivores and plant pathogens than the respective substrate [1]. *Bx10/11/12/14* are part of a small gene cluster containing two additional and so far uncharacterized OMT genes (*ZmFOMT2* and *ZmFOMT3*). Despite high sequence similarity to *BX10/11/12/14*, recombinant ZmFOMT2 and ZmFOMT3 were not able to accept benzoxazinoids as substrate. Instead, both ZmFOMT2 and ZmFOMT3 showed OMT activity with different flavonoids such as naringenin and apigenin. Purification of the enzyme products followed by NMR structure elucidation revealed that the different flavonoids were specifically methylated on the hydroxyl group at position 5 of the A-ring. A transcriptome analysis showed that *ZmFOMT2* was strongly induced after infestation with *Bipolaris maydis*, a maize pathogen that causes Southern corn leaf blight disease (SLB). In addition, the transcriptome revealed a second highly induced putative OMT gene (*ZmFOMT4*) with similarity to *ZmFOMT1*, a previously characterized OMT that methylates the B-ring hydroxyl groups of different flavonoids [2]. Indeed, recombinant ZmFOMT4 showed also activity with flavonoids, but specifically methylated the hydroxyl group at position 7 of the A-ring.

To investigate whether methylated flavonoids are actually produced after fungus infestation in maize, we analyzed the content of unmethylated and methylated flavonoids in SLB-infested and non-infested maize plants using LC-MS/MS. In comparison to the control plants, fungus infested plants showed a significant induction of the flavonoid pathway per se (expressed in a higher content of the first intermediate naringenin chalcone) and a significant accumulation of different *O*-methylated flavonoids mainly formed from apigenin and the apigenin-derivative scutellarein (such as 7-methylapigenin, 7-methylscutellarein, 5,7-dimethylscutellarein). Our data indicate that ZmFOMT2 and ZmFOMT4 in combination are likely responsible for the methylation of flavonoids *in vivo*. Moreover, we speculate about an important role of methylated flavonoids in the defense of maize plants against pathogenic fungi.

[1] Meihls, L. N. et al. (2013) *Plant Cell* 25: 2341-2355
[2] Zhou, J. M. et al. (2008) *Pharmaceutical Biol.* 46: 26-34

Talk 9

At the edge: How the macronutrient sulfate is used to modify the secondary metabolites salicin and salirepin in poplar

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Due to their sessile way of life, plants have to adjust their metabolism to a variety of changing abiotic and biotic stresses. While the term abiotic stress comprises among others nutrient- and water availability, biotic stress describes the interactions of the plant with other living organisms as for instance herbivores and pathogens. For a long time the research on these two kinds of environmental stresses and the resulting metabolic adaptations of the plants were strictly separated. In this study we identified two compounds that represent a merging point between classically termed secondary metabolites and the primary metabolite sulfate, a macronutrient that is often limiting and whose lack can lead to plant stress responses. These two compounds, salicin-7-sulfate and salirepin-7-sulfate, are sulfated versions of the salicinoids salicin (2-hydroxybenzyl alcohol glucoside) and salirepin (2,5-dihydroxybenzyl alcohol glucoside) and were isolated from the model tree species *Populus trichocarpa*. In general, salicinoids are defense metabolites acting against herbivores and pathogens. They are characteristic for the *Salicaceae* (poplars and willows) and defined by a chemical core structure consisting of a 2-hydroxybenzyl alcohol moiety bound to a β-D-glucopyranose. We aimed to identify and characterize enzymes responsible for the sulfation of salicin and salirepin. Sulfotransferases (SOT), which are able to transfer a sulfuryl group from the cofactor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to different chemical compounds, have been described in a few plant species. Using a transcriptomic approach, we identified a *SOT* candidate gene (*PtSOT1*) in *P. trichocarpa*. When incubated *in vitro* with salicin and salirepin as substrates, recombinant PtSOT1 produced salicin-7-sulfate and salirepin-7-sulfate. Moreover, RNAi-mediated down regulation of *PtSOT1* in poplar resulted in a decreased accumulation of salicin-7-sulfate and salirepin-7-sulfate, indicating a significant contribution of *PtSOT1* to the formation of these compounds *in vivo*. We are currently using these transgenic trees in bioassays with the generalist herbivore *Lymantria dispar* to investigate the biological relevance of salicin-7-sulfate and salirepin-7-sulfate in poplar defense. In future experiments we want to elucidate the role of the two sulfated compounds as a potential internal sulfate storage pool, and the ability of the plant to remobilize sulfate from salicin-7-sulfate and salirepin-7-sulfate.

Talk 10

Forisomes – possible key players in legume defense against aphids

Maria K. Paulmann[1,2], Grit Kunert[1], Ralf Oelmüller[2], Jonathan Gershenzon[1], Alexandra C.U. Furch[2]

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Every gardener knows them as pests on their plants: aphids – these small insects that appear in different colors such as green, red or black. *Acyrthosiphon pisum* is a legume specialist which is known for its different host races. Each host race is specialized on one or just a few legume species. For example, the Trifolium host race can only develop on *Trifolium pratense*. However, all *A. pisum* host races can feed on the universal host plant *Vicia faba*. Aphids feed from the phloem sap of their host plant and can cause great damage to agriculture through transmission of phytopathogens such as viruses and/ or explosions in aphid population size.

In response to the possible loss of phloem sap, for example through phloem feeding insects or damage to the vascular system, plants have evolved mechanisms to occlude their sieve elements. This occlusion is mediated via callose deposition and phloem (p)-proteins. In the *Papilionoids* of the Fabaceaen family a special form of p-protein – the forisome - has developed. It can reversibly change its conformation in a Ca^{2+}- instead of an ATP-dependent manner. The exact functionality of forisomes has been subject to frequent discussions and previously been indicated to play a role in legume defense against phloem feeding insects.

We investigated the influence of aphid feeding on forisome reactivity in the host plants *V. faba* and *T. pratense.* Comparing the effect of different *A. pisum* host races showed that the suppression of forisome reactivity does depend on the plant – host race interaction and may play a role in *A. pisum* host race maintenance.

Talk 11

Novel zwitterionic metabolites from marine diatoms

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Zwitterions are characterized by the presence of both a positive and a negative charge within one molecule.

They play an important role in the environment, since they are involved in the modulation of the global sulphur cycle in the atmosphere and since they mediated many interactions among marine species.

The identification and classification of zwitterionic metabolites has been problematic until our development of novel chromatographic and mass spectrometric methods. These analytical methods show the presence of many zwitterions in microalgae that have not been recognized or characterized previously. In this talk, I will present how liquid chromatography coupled with mass spectrometry is utilized to assign novel hitherto unknown components in the “zwittermetabolome” of diatoms. The physiological functions of novel key metabolites is introduced and ecological implications are discussed. I report studies on three diatom species, *Phaeodactylum tricornutum*, *Skeletonema costatum* and *Thalassiosira weissflogii* that have emerged as model organisms in phycological studies.

Talk 12

Uncoupling pre- and post-pollination in *Nicotiana attenuata* to evaluate the potential and actual outcrossing of different pollinators

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Nicotiana attenuata, a self-compatible wild tobacco species, has a broad community of flower visitors ranging from day active hummingbirds and bees to night active hawkmoths. Since flower visitation per se does not guarantee efficient pollination, the aim of this study was to evaluate the pollinator efficiency in terms of outcrossing for different floral visitors of *N. attenuata*. The most common measures of pollinator efficiency such as pollen uptake rates, number of conspecific pollen grains deposited or seed set, neglect one important step between pollination and fertilization, i.e. post-pollination pre-zygotic mate selection. By just taking pollen deposition or seed set as a measure the importance of a pollinator for a plant´s reproduction might easily be over- or underestimated. Therefore, in this project we uncouple pre- and post-pollination events to gain a better understanding of potential and actual outcrossing resulting from pollination by different floral visitors.
For this, we conducted experiments with transgenic plants disrupted in post-pollination mate selection (irACO) in comparison to control empty vector plants (EV). For our experiments, we used non-emasculated flowers to allow for natural selfing rates. To test how much outcross pollen is siring seeds when EV and irACO flowers are allowed to naturally self, we added low and high loads of outcross pollen to the stigma and genotyped the offspring using microsatellite markers. Applying low amounts of outcross pollen resulted in outcrossing rates below 3%, while high loads of additional outcross pollen resulted in 30 to 40% outcrossing. For evaluating the potential and actual outcrossing by different floral visitors, we planted pairs of EV and irACO surrounded by four accessions (paternal genotypes) in natural and semi-natural conditions for open pollinations and controlled hand-pollinations. Seeds produced after pollinator visitation were used for genotyping to estimate outcrossing rates in irACO (reflecting potential outcrossing) and EV flowers (reflecting actual outcrossing after post-pollination mate selection) by using microsatellite markers. In the plant´s natural habitat flowers of EV as well as irACO visited by day pollinators did not contain any outcrossing. Capsules from flowers visited by *Manduca sexta* in semi-natural tent experiments contained up to 70% of outcrossed seeds. Up to all four paternal genotypes sired seeds but the percentage of the paternal genotypes per capsule was highly variable between the replicates. However, in flowers visited by *M. sexta* the potential outcrossing (irACO) consistently tend to be higher than the actual outcrossing (EV), proving mate selection after insect pollination in a semi-natural environment. This in combination with the high variation of pollen donor identity suggests that *M. sexta* is offering a highly diverse pool of pollen mates in comparison to other pollinators, and thus giving a N. attenuata plant the opportunity to choose from a larger number of mates and to realize multiple paternity. To our knowledge, this study is the first example of dissecting potential and actual outcrossing by different pollinators in a self-compatible plant species.

Talk 13

Low abundances of irMPK4 plants in population increase total population yield, but only without AMF interactions

Talk 14

Mass spectrometry imaging on plants - the ups and downs of method development

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In the wake of recent technical developments, mass spectrometers offer new possibilities in performing experiments to localize and visualize biologically active molecules. The label-free nature of such mass spectrometry imaging experiments allows for the simultaneous tracking of multiple molecules.

As most research and method development in mass spectrometry (MS) imaging is used for with medical research and subsequent analytics. An application for samples other than animal tissue sections faces multiple challenges. Amongst others, surface topography and mechanical robustness of samples (e.g. of plant and insect origin) represent a crucial challenge.

Building a custom-made laser ablation electrospray ionization (LAESI) source, I integrated a distance sensor to measure the surface topography prior to the actual MS experiment. Thus, the resulting ion source was able to operate on samples with a pronounced three-dimensional shape.

Basic functionality and viability of the concept will be demonstrated on cotton (*Gossypium hirsutum*) leaves and stems. The oil glands of most cotton species contain high concentrations of gossypol and similar compounds which makes them a useful sample for validation and control

Nevertheless, certain challenges arise concerning a stable and reproducible sample measurement. Current draw-backs and putative solution approaches will be presented to overcome these challenges and allow for a more widespread use of this new ion source system.

Posters

#129812798

Poster 1

Arabidopsis thaliana mutants: A versatile tool to investigate the influence of polygalacturonase-inhibiting proteins on the beetle *Phaedon cochleariae*

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The herbivorous mustard leaf beetle *Phaedon cochleariae* feeds on brassicaceous plants and possesses various digestive carbohydrases targeting plant cell wall polysaccharides. Amongst those, polygalacturonases (PGs) hydrolyse the cell wall polysaccharide pectin. Plant-derived, cell wall-associated polygalacturonase-inhibiting proteins (PGIPs) counteract microbial PGs and thus contribute to the plant´s defence against phytopathogens. However, direct interactions between beetle PGs and plant inhibitory proteins have not yet been investigated. We performed interaction studies of *P. cochleariae* PG family members with crude cell wall protein extracts of the beetle's food plant Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Putative PGIPs and other leucine-rich repeat (LRR) proteins were identified by MS/MS representing candidates for beetle PG inhibition. Heterologous expression of candidate proteins allows for the characterisation of their specificity and inhibitory activity towards beetle PGs in vitro. Both PGs and PGIPs belong to multigene families that are believed to have been shaped by an evolutionary arms race. The number of PGIPs in brassicaceous plants ranges from two in *Arabidopsis thaliana* to nine and 16 in Chinese cabbage and rapeseed (*B. napus*), respectively. The oligophagous *P. cochleariae* can be reared on *A. thaliana* and, in contrast to Chinese cabbage, both AtPGIPs have been extensively characterised. The variety of knockout mutants and versatile molecular tool box available motivated us to include *A. thaliana* to investigate the potential role of PG – PGIP interactions in vivo. We used mutants lacking either AtPGIP1 or AtPGIP2 to analyse (i) beetle life history and beetle PG regulation in the presence or absence of putative inhibitors and (ii) the regulation of plant PGIPs in response to beetle feeding. Integrating data from both the model *A. thaliana* (easy to manipulate) and the beetle's food plant Chinese cabbage (ecologically more relevant) will shed light on the role of plant PGIPs in defence against herbivorous beetles in general.

Poster 2

Chemical characterization of *Nepenthes x ventrata* extrafloral nectar

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The carnivorous pitcher plant *Nepenthes* x *ventrata (N. alata x N. ventricosa)* is a natural hybrid endemic of the Philippines. It lives on soils with nutrients (nitrogen) deficiency. These plants have evolved to attract, kill, digest and uptake nutrients from captured insects. There is lacking information regarding the preference capture of insect's species for *N.* x *ventrata*. Nevertheless, 97% of insects captured in *N. alata* in the field are ants. In terms of defense, many plants under herbivory attack attract ants for indirect defense by secreting extrafloral nectar (EFN). Examples are the well-known ant-plant interactions of *Vachellia collinsi*, *V. chiapensis*, and *V. farnesiana* plants. In case of *V. collinsii* and *V. chiapensis,* plants and ants live in close mutualisms in so-called myrmecophytic interactions. In non-myrmecophytic interactions, the ants appear on demand such as in *V. farnesiana*. Here, we characterized the chemical composition of the EFN from *N. x ventrata* from the chemical point of view. The scrutiny is based on sugar composition (glucose, fructose and sucrose), amino acids (AA), and carbon/nitrogen ratios as well as fluorescent properties. The C/N ratio in *N. x ventrata* EFN was found to be 7.5 to 8.4 times higher than in myrmecophytic *Vachellia* EFN and two times higher than in EFN of the non-myrmecophytic *V. farnesiana*. The total AA content in *N. x ventrata* was 20 times lower compared with the *Vachellia* spp. The production of protein and free AA implicates a high cost investment for the plants. Such investment is detrimental for plants with nitrogen deficiency. The EFN in *N. x ventrata* is potentially produced only to attract, catch and finally digest ants and not to recruit them as secondary defense, as it is the case of the myrmecophytic *V. collinsii* and *V. chiapensis*. Further research on the ecological implications of the EFN production in carnivorous plants has to been done in order to understand and interpret the ant-plant interaction.

Post 3

DNA of sexy perfume repelling neighbours

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Sexual attraction through sex pheromones is best studied in moths, where females emit species-specific multi-component blends. The species-specificity stems from the presence/absence and the ratios of the different components. Some components are well known to be either attractive or repellent for different species. This is for example the case for acetate esters, which are present in numerous species and act as repellent for species that don't produce it. Despite this knowledge, the genes involved in the acetate production is still a mystery.

This project aims to identify the gene(s) involved in acetate production in moth sex pheromones. We will focus on the specialist moth *Heliothis subflexa,* in which geographic variation of acetate esters has been found. In areas where both *H. subflexa* and the closely related *Heliothis virescens* are present, *H. subflexa* females produce more acetate to repel *H. virescens* males. Previous studies have already found two major QTL, chromosome 20 and 28, that are involved in the acetate production in *H. subflexa*.

The main candidate genes in these QTL are acetyl transferases and esterases. We have recently identified a transposable element in an esterase of chromosome 28 that decreases the level of acetates. We are currently knocking out this esterase by CRISPR-Cas9 to confirm this result. This would be the first identification of a single gene that could explain at least some of the acetate variation.

Poster 4

The role of plant beta-glucosidases and beta-glucosidase-aggregating factors in BXD activation

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β-Glucosidases 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 activation of defense compounds. 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 plants, 1,4-benzoxazin-3-one derivatives (BXDs) like DIMBOA-Glc, are stored as glucosides and activated by plant β-glucosidases. Two β-glucosidases (ZmGlu1 & ZmGlu2) have been described to hydrolyze DIMBOA-Glc. *ZmGlu1* & *ZmGlu2* form a distinct gene subfamily with six other *ZmGlu* genes (*ZmGlu3-8*). Since *ZmGlu3-8* share 70-80% similarity with *ZmGlu1* & *2*, it is likely that they have similar catalytic activities. In this study, we investigated the expression levels of *ZmGlu1-8* in undamaged and herbivore-damaged leaves and roots of maize seedlings and biochemically characterized the recombinant enzymes. The substrate specificities were assayed with artificial β-glucosidase substrates as well as different BXDs.

Some maize lines possess only marginal soluble β-glucosidase activity after tissue disruption. In these lines, β-glucosidases interact with a protein called β-glucosidase aggregating factor (BGAF) and form insoluble protein complexes. BGAF-β-glucosidase aggregation has been suggested to protect β-glucosidases from proteolytic degradation in the insect gut. However, the function of BGAFs, even in the context of β-glucosidase stabilization, is yet unclear. So far it's known that BGAF1 & BGAF2 interact with ZmGlu1 & ZmGlu1. Using gel shift assays, we tested the potential interactions of the other purified recombinant BGAF proteins with the different recombinant ZmGlu proteins.

Poster 5

Effects of the main secondary metabolites of Physalis plants on a specialist and a generalist species of Lepidoptera

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Specialisation on Physalis plants requires the ability to tolerate withanolides, the main secondary metabolites of these plants that are known to have feeding deterrent, cytotoxic and immune inhibiting properties. However, previous studies demonstrated that *Heliothis subflexa* directly and indirectly benefits from immune modulating activities of withanolides from its host plant *Physalis peruviana*. These findings raise questions on the mechanisms by which *H. subflexa* overcame the inhibitory effects of withanolides.

To the best of our knowledge, nothing is known about the fate of withanolides in the caterpillar body. Withanolides are a group of C_{28} steroids built on an ergostane skeleton. The high reactivity of the unsaturated carbonyl system of these compounds suggests an activation after ingestion in the insect gut by e.g. P450 enzymes, which are known to be involved in metabolite detoxification in other lepidopteran species. Preventing this activation in *H. subflexa* larvae could explain their apparent tolerance to withanolides.

As one part of this PhD project, feeding assays are planned in which *H.subflexa* and the closely related generalist species *Heliothis virescens* are fed with defined amounts of purified withanolides to elucidate their potential metabolism or metabolic circumvention. Subsequent analysis of the faeces, gut and the rest body via LC-MS will allow us to detect differences in withanolide metabolism between both species. Afterwards, further experiments will be performed to identify the mechanisms that cause the expected differences in withanolide conversion.

Poster 6

Secondary Metabolites in Seed Development of *Musella lasiocarpa*

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Musella lasiocarpa, a member of the Musaceae (Fig.1), is an endangered endemic banana species in Southwestern China.[1] The plant has no importance as food source but it is well-known as an ornamental plant. We were interested in the built-up of secondary metabolites in the seeds during their development to complement the knowledge on developmental biology of *Musa* species.[2] Seeds of different developmental stages were sampled and analyzed for their metabolic profiles by high performance liquid chromatography coupled with high-resolution electrospray ionization mass spectrometry (HPLC-HRESIMS) and fluorescence detection (FLD). The identity of metabolites was elucidated by means of nuclear magnetic resonance spectroscopy (NMR) which eventually enabled us to construct a timetable of emerging metabolites formed during seed development.

Figure 1: *Musella lasiocarpa* (Musaceae)

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Poster 7

Molecular evolution of arylsulfatases involved in glucosinolate detoxification in the flea beetle genus *Psylliodes*

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The cosmopolitan genus flea beetle genus *Psylliodes* (Chrysomelidae, Galerucinae, Alticini) comprises more than 200 species which are associated with host plants in 22 different families. About 47% of the species with known host plants are specialized to feed on Brassicaceae, including the cabbage stem flea beetle, *Psylliodes chrysocephala*, an important pest of winter oilseed rape in Europe. Brassicaceae plants defend themselves from herbivores through the glucosinolate-myrosinase defense system. Upon tissue damage, glucosinolates are hydrolyzed by the thioglucosidase enzyme myrosinase, forming isothiocyanates which deter non-adapted herbivores. Recent studies showed that *P. chrysocephala* partially prevents glucosinolate activation by conversion to desulfo-glucosinolates using glucosinolate sulfatase enzymes. Phylogenetic analyses of coleopteran arylsulfatases revealed that glucosinolate sulfatases evolved by duplications of the arylsulfatase 4 (*Sulf4*) gene in *P. chrysocephala*. Here, we analyzed the molecular evolution of the arylsulfatase gene family in the genus *Psylliodes*. In a phylogenetic analysis of 51 *Psylliodes* species associated with different host plant families, most Brassicaceae-feeding species grouped together in a separate and well-supported clade, except for two species (*P. kiesenwetteri* and *P. vehemens*), which grouped together with non-Brassicaceae feeding species in the basal clade. We then analyzed the molecular evolution of arylsulfatase genes identified in the transcriptomes of eight *Psylliodes* species including those of *P. chrysocephala*. This analysis revealed a specific expansion of *Sulf4* genes in Brassicaceae-feeding species, except for *P. kiesenwetteri*, which only possesses one *Sulf4* gene. Next, we will determine whether the diversified *Sulf4* genes in other Brassicaceae-feeding *Psylliodes* species encode glucosinolate sulfatases. This study provides insights into the evolution of a biochemical adaptation, which enables a specialized herbivore to overcome the chemical defence in its host plants.

Poster 8

Solving the yellow mystery of *Papaver nudicaule* with an integrated -omics approach

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Indole alkaloids are a widespread group of secondary metabolites with huge structure variety. Nudicaulins, yellow flower pigments of *Papaver nudicaule* (Island poppy), are unique representatives of this compound class, combining structural elements of indole and flavonoids. Previous studies identified indole and pelargonidin glucosides as final precursors. The aim of our work is to understand the biosynthesis of the nudicaulins based on a four-pillar approach: NMR studies on the pigment chemistry, UPLC-HRMS and LC-UV/ Vis based metabolomics, transcriptomics, and DIGE-based quantitative proteomics.

Firstly, the development of the *P. nudicaule* flowers was divided in five stages. Therein, the most important genes and their products involved in the shikimate/ phenylpropanoid/ polyketide as well as indole biosynthetic pathway were identified by transcriptomic, proteomic, and metabolomic analyses. Relevant metabolites were semi-quantified. Additional experiments showed that the final nudicaulin formation from indole and pelargonidin glucosides is also possible *in vitro* under acidic pH conditions. This raises the question about the exact mechanism of this reaction *in vivo,* which is a work in progress.

Based on transcriptomic, proteomic, metabolomic, and NMR studies we propose a biosynthetic pathway of nudicaulins in yellow petals of *P. nudicaule*. This opens new insights into this unique class of indole alkaloids.

Poster 9

Cerura vinula: Salicinoid metabolism in a specialist herbivore

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Plants of the *Salicacea* genus have in common that they utilize phenolic glycosides, so called salicinoids, as protection against leaf chewing herbivores. The caterpillar *Cerura vinula* only feeds on poplar and willow trees that belong to the family of *Salicacea*. For this reason, *Cerura vinula* is viewed as a specialist herbivore that is adapted to the salicinoid defense. Taking advantage of chemical and biochemical techniques we want to trace the salicinoid metabolism pathway, the location of transformation and the involved enzymes.

To gain insights into the salicinoid metabolism the caterpillars were put on a specially designed diet. It consisted either of one salicinoid in large excess or one ^{13}C labelled compound, applied together with fresh poplar leaves. As a next step, we are going to identify the new compounds formed in the caterpillar by MS and NMR techniques.

To determine the location and mechanism of the transformation we started with a dissection of the caterpillar and a check of the tissue pH value. Afterwards, we incubated the midgut, hindgut and salivary gland tissues with salicin as model substrate and analyzed the transformation products by LC-MS. The transformation products resulted from deglycosilation of the substrate and consecutive oxidation and conjugation of the aglycon. With the gained knowledge we then aimed to identify the enzymes, which are involved in the metabolism. We successfully proofed the existence of glucosidases in the midgut by isoelectric focusing and incubation of the gel with the model substrate 4-methylumbelliferyl *β-D*-glucopyranosid. Additionally, we performed RNA sequencing on the caterpillar tissue. The transcripts and enzymes involved in the salicinoid metabolism are currently analyzed. During our studies we could determine the mid gut as the place for the deglycosilaton, oxidation and conjugation of salicinoids. Further we identified a new for *Cerura vinula* undescribed metabolite and proofed the presence of *β*-glucosidases in the mid gut.

Poster 10

Who's There? Chemical Perception of Microbes by the *Arabidopsis* Root

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Plant roots play a crucial role in perceiving chemicals released from microbes in the rhizosphere. These chemicals serve as the mediator for plants and microbes to communicate with each other. One prevalent system in plants to identify these different chemical compounds is through calcium signaling. Once a receptor in roots is activated by a microbe-released chemical, a specific calcium signature initiates specific downstream phosphorylation events. Therefore, plants can recognize the microbe nearby as friend or foe, thus respond to it accordingly. Previously, a key molecule, cellotriose, was found to be produced and released by the beneficial fungus *Piriformospora indica*. Cellotriose triggers a rapid calcium elevation in root cells within 90 second. However, up to date, the responsible receptor(s) for cellotriose perception and the downstream events remain unknown. In this study, we identified two ethyl methanesulfonate (EMS)-induced A*rabidopsis* mutants that responded to cellotriose differently from wild-type. These two mutants could provide insight into the molecular mechanism of cellotriose perception.

Poster 11

Decoding the odorant receptor repertoire of the hawkmoth *Manduca sexta*

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Manduca sexta expresses 72 odorant receptors (ORs) in its antennae. However, only MsexOR1 – the pheromone receptor detecting the female-produced sex pheromone bombykal – has been deorphanised till date. We aim to find ligands for more ORs, and investigate how the moth encodes odor cues in its receptor repertoire. Using genetic tools we heterologously expressed individual moth ORs in the antennae of the vinegar fly *Drosophila melanogaster*, and electrophysiologically tested olfactory responses to a set of 80 chemically diverse and ecologically relevant odorants. We found a broadly-tuned OR, MsexOR36 that gives strong responses to terpenes and aliphatic esters. These compounds are found in volatile profiles of hawkmoth pollinated flowers, suggesting a role of MsexOR36 in foraging behavior. In addition, we found ORs that have very different response profiles and are more narrowly-tuned. Our results provide the first glimpse of plant odor coding by *Manduca sexta* and is a step towards unravelling the impact of single odorant receptors in moth behaviour.

Poster 12

Mechanisms of Rhizobia tolerance to Aluminium stress-An overview

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Common bean is a common source of proteins for most Kenyans. However, in the recent years, its production has been very low due to poor soil fertility. Inorganic fertilizers are expensive and out of reach of poor farmers. Inoculation of common beans with effective *Rhizobia* which convert free atmospheric N_2 to NH_4^+ utilizable by plants is a better alternative. However, their growth, proliferation and symbiotic potential are restricted by high aluminium toxicity in these soils. This study aims at determining mechanisms in which indigenous rhizobia are able to tolerate alum toxicity so that this mechanism may be transferred to effective but susceptible rhizobia for inoculation in Western Kenya soils.

Poster 13

Single-nucleus transcriptomics of olfactory sensory neurons and support cells in the *Drosophila* antenna

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Olfaction is a key sensory modality in insects. In flies, odors strongly influence feeding, reproductive and navigational behaviors. The third segment of the antenna in flies is responsible for detecting odors via numerous hair-like structures called *sensilla*. Each sensillum houses one or several odor- and pheromone-sensitive neurons, which detect volatiles and subsequently relay this information to the brain. These sensory neurons are canonically divided into 'types' based on anatomical location and expression of one-or-few olfactory receptors on their dendritic membrane. However, little is known about the heterogeneity of antennal cells. Are all OSNs self-similar, aside from the expression of specified surface receptors? Do supporting and glial cells, which make up the majority of the antenna, participate in the odor detection process? What are the unique molecular properties of each cell type? To gain a more complete picture, we sequence each individual cell's transcriptome separately using single-nucleus RNA sequencing. This is achieved by isolating antennal nuclei, encapsulating each nucleus within a reagent droplet using microfluidics, and preparing a cDNA library capable of being demultiplexed to reveal singular cell differences in mRNA expression. Understanding the transcriptomic profiles of these cells at an individual level will expand our understanding of the signal transduction event, help reveal new cell-specific genes for further study, and create a molecular atlas of antennal cells. Furthermore, single cell transcriptomics will allow us to characterize the poorly described *support cells*, which are thought to be indispensable for successful olfactory signaling and behavior.

Poster 14

Beetle-induced plant response leads to a shift in feeding preference of *Lymantria dispar* caterpillar

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Herbivorous insects encounter drastic variations of primary and secondary metabolites even within one single tree crown. Recent studies have shown that insect herbivores often exhibit a feeding preference for a specific leaf age over another and that they are able to distinguish between damaged and undamaged leaves. However, there is a lack of knowledge on the combined effect of leaf age and damage status. Since herbivores induce direct plant defenses, this may influence the feeding behavior of subsequently herbivores. In this study we examine whether indirect food competition leads to a shift in the feeding preference of a generalist insect herbivore for specific leaf ages. In a choice assay generalist gypsy moth (*Lymantria dispar*) caterpillars could select between young, middle aged and old leaf discs from young black poplar (*Populus nigra*) trees. *Prior* to the choice assay the trees experienced herbivory by poplar leaf beetles (*Chrysomela populi*), which were allowed to feed either on young, middle aged or old leaves to mirror indirect food competition. We analyzed sugars, amino acids and phytohormones as well as major defense compounds like salicinoids, flavonoids and protease inhibitors to determine changes between different leaf ages and the effect previous feeding damage has on *P. nigra* phytochemistry. In undamaged trees, *L. dispar* preferred old leaves over young and middle aged leaves. However, when the old leaf pool was previously damaged*, L. dispar* showed generally less feeding activity and switched its feeding preference for the young leaf pool. We could show that previous feeding damage on plants by a specialist herbivore lead to a shift in the feeding preference of a generalist herbivore. This study provides important insights in how insects compete within a single tree crown.

Poster 15

Deorphanization of chemosensory neurons in *Drosophila melanogaster*

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Chemosensation is essential for the survival of insects. Activities like search for food, mating, and egg laying are carried out by the fruit fly, *Drosophila melanogaster* by sensing various chemical cues with the help of olfaction and gustation. These chemical cues are conveyed to higher brain centers via diverse olfactory sensory neurons (OSNs) and gustatory sensory neurons (GSNs). However, few of these OSNs and GSNs remain orphan. We aim to deorphanize some of these neurons in the *D. melanogaster* olfactory organs, thereby finding their natural ligands and their contribution in the behavioural output. Initially, we will focus on the orphan olfactory receptor (Or33a) that is coexpressed with Or56a, which is in turn responsible for the detection of the deterring compound geosmin. Additionally, we will use an olfactogenetics approach to deorphanize three 'sweet' gustatory receptors (Gr5a, Gr64b, Gr64f) conspicuously expressed in the olfactory organs of the fly. We will use 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 fly's chemosensation by identifying natural ligands for orphan neurons and their role in the overall behaviour.

Poster 16

Nascent secondary metabolites: Evolution of early iridoid synthesis in Nepeta

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The seco-loganic pathway is a well-studied pathway in plants responsible for creating ~3000 monoterpenoid indole alkaloids, many used in medicine. The early iridoid pathway creates the initial iridoid scaffold, 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 iridoids. The O'Connor lab showed that Nepeta ISY has convergently evolved from an alternative ancestor. When the precursor gene to ISY, progesterone 5 beta reductase (P5βR), was tested from a non-iridoid producing Nepetoideae (Hyssopus officinalis), it was shown to have iridoid producing activity in vitro. Thus, the function of the in vivo pathway, and its potential evolutionary path, were studied in this project. We identified the early enzyme homologues in Nepeta and Hyssopus, including geraniol synthase (GES), geraniol-8-hydroxylase (G8H) and hydroxy-geraniol oxidase A (HGOA). Transcriptomic data suggests that low gene expression plays a role in metabolite landscape for each species. Furthermore, in vitro biochemical assays on purified enzymes suggests much lower enzyme efficiency of non-Nepeta early iridoid pathway genes. This data supports the idea that a latent, but mostly inactive, pathway in non-iridoid producing Nepetoideaes may have provided the landscape for the resurgence of iridoid production in Nepeta.

Poster 17

Towards FDR estimation in computational metabolomics

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Identification of metabolites from biological samples using mass spectrometry data is an ongoing topic in metabolomics, with the majority of metabolites still being unknown.
Since manual interpretation of large scale data sets is impractical, we recently presented a computational method for the automated analysis of small molecule fragmentation data – SIRIUS/CSI:FingerID.

Conventionally, researchers would try to match an experimental tandem mass spectrum against a spectral database. Converting the MS/MS spectrum into a molecular fingerprint using machine learning allows our method to match against (much larger) structural libraries instead.

As no automated metabolite identification tool is able to correctly identify 100% of molecules in a query, it is important for user and developer alike to be able to assign a “confidence level” for each output.
This is usually reported in the form of false discovery rate estimations or other statistic measures.
We show how we significantly improved the ability of CSI:FingerID to assign a confidence score to a given output and how it could influence high throughput, whole dataset identifications.

Poster 18

Terpene emission in a combination of drought stress and Methyl Jasmonate treatment in a conifer species *Picea glauca*

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Picea glauca is a conifer species emitting monoterpenes and sesquiterpenes which are involved in important ecological function, such as direct or indirect defences against herbivore.

By spraying 2 years old *P.glauca* trees, with Methyl Jasmonate (MeJA), we simulated a phytohormone reaction occurring after herbivore attack, in order to evaluate the terpene emission response. Drought episodes are supposed to increase in the frame of the future climate change. We wanted to investigate how drought stress can affect the terpene response by withholding water for two weeks. We found that the monoterpenes and sesquiterpenes emission reduced when the MeJA treatment was combined with the application of drought stress. We analysed the terpenes content in the needles, but we did not find difference in the composition and amount of those compounds. The possibility to develop traumatic resin ducts in needles was also evaluated, by counting the length percent of the ducts in the total length of the needle. This experiment gives a new point of speculation on how drought stress can influence the emission of terpene and the interaction between *P.glauca* and herbivores.

Poster 19

Differential equation based minimal model describing metabolic oscillations in *Bacillus subtilis* biofilms

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Biofilms offer an excellent example of ecological interaction among bacteria. Temporal and spatial oscillations in biofilms are an emerging topic. In this paper we describe the metabolic oscillations in *Bacillus subtilis* biofilms by applying the smallest theoretical chemical reaction system showing Hopf bifurcation proposed by Wilhelm and Heinrich in 1995. The system involves three differential equations and a single bilinear term. We perform computer simulations and a detailed analysis of the system including bifurcation analysis and quasi-steady-state approximation. We also discuss the feedback structure of the system and the correspondence of the simulations to biological observations. Our theoretical work suggests potential scenarios about the oscillatory behaviour of biofilms and also serves as an application of a previously described chemical oscillator to a biological system.