IMPRS

Exploration of Ecological Interactions with Molecular and Chemical Techniques

17th IMPRS Symposium

March 13-14, 2018

Old Castle, Dornburg

Program

Tuesday, March 13th, 2018

08:00	*Departure, central bus stop (opposite Jena Paradies train station)*
08:45	**Welcome and Announcements** (Großer Kaisersaal) Prof. Jonathan Gershenzon, spokesperson of the IMPRS
09:00	**Plenary Lecture 1** (Großer Kaisersaal) Evolution of olfactory systems in Drosophilids *Dr. Lucia Prieto-Godino, The Francis Crick Institute, London, UK*, p.2 *Chair: Mohammed A. Khallaf*
10:00	*Coffee break (Rittersaal)*
	1st talk session (Großer Kaisersaal)**,** *chair: ??*
	Part 1 - Studying olfactory systems in *Drosophila*
10:20	1 - Finding a partner: Sexual communication in *Drosophila* *Mohammed A. Khallaf (HAN)*, p.4
10:40	2 - Evaluation of the DREAM method for high-throughput deorphanization of chemosensory receptors *Sarah Körte (HAN)*, p.5
11:00	3 - Time to learn: changing hedonic valence of an odor with experience *Florencia Campetella (HAN)*, p.6
	Part 2 - Applied and theoretical metabolomics
11:20	4 - Identification, physiological and ecological function of zwitterionic metabolites in marine diatoms *Simona Fenizia (FSU)*, p.7
11:40	5 - Towards FDR estimation in computational metabolomics *Martin Hoffmann (FSU)*, p.8
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*
	2nd talk session (Großer Kaisersaal), chair: ?? **Vegetarian insects – coping and evolving with plant diets**

15:15	6 - Molecular evolution and ecology of cotton secondary metabolite detoxification in Heliothine moths *Corinna Krempl (HEC)*, p.9
15:35	7 - Interaction of beetle polygalacturonases with plant cell wall proteins Wiebke Häger (HEC), p.10
15:55	8 - Overcome glucosinolates and radiate: How host shifts to Brassicaceae have led to species diversification Matilda Gikonyo (HEC), p.11
16:15	9 – The sweet life: Mechanistic parallels between osmoregulation and detoxification in *Bemisia tabaci* Michael Easson (GER), p.12
16:35	**IMPRS Special Topic Talk** TReND in Africa: Bridging the gap towards a truly global scientific community *Dr. Lucia Prieto-Godino, The Francis Crick Institute, London, UK*, p.13
17:15	Bus returns to Jena
18:15	Dinner with guest speakers – Zur Noll
19:00	Discussions with guest speakers and MPICE scientists about collaborative projects
20:30	End of day 1

Wednesday, March 14th, 2018

08:00 *Departure, central bus stop (opposite Jena Paradies train station)*

09:00 **Plenary Lecture 2** (Großer Kaisersaal)
Arbuscular mycorrhiza development and function
Prof. Caroline Gutjahr, TU Munich, p.3
Chair: Chhana Ullah

10:00 *Coffee break (Rittersaal)*

3rd talk session (Großer Kaisersaal), chair: ??
How do plants cope with insects, viruses, fungi and drought?

10:20 10 - Reactions of susceptible and tolerant barley genotypes after barley yellow dwarf virus infection
Maria Paulmann (GER), p.14

10:40 11 - TOC1 function in shoots, but not roots, mediates *Nicotiana attenuata*'s drought responses in nature
Henrique Valim (ITB) p.15

11:00 12 - The role of the glucosinolate-myrosinase defense system in the interaction between *Arabidopsis thaliana* and *Sclerotinia sclerotiorum*
Jingyuan Chen (GER), p.16

11:20 13 - NaMPK4 plays a role in *Nicotiana attenuata*'s growth responses to neighbors
Erica McGale (ITB), p.17

11:40 14 - Specificity of black poplar defense responses to various insect herbivores
Thomas Fabisch (GER), p.18

12:00 Lunch

13:00 **Poster talks 2** (Großer Kaisersaal)
Even numbers (1 slide and 1 min/poster)

13:15 **Poster session 2** – even numbers (Rittersaal)

14:40 *Coffee break (Rittersaal)*

4th talk session (Großer Kaisersaal), chair: ??
Selected topics from plant pollination and beetle defense

15:00 15 – Finding the best pollinator by uncoupling pollinator attraction and post-pollination mate selection in *Nicotiana attenuata*
Julia Bing (ITB), p.19

15:20 16 - A juvenile leaf beetle cytochrome P450 is involved in iridoids biosynthesis
Nanxia Fu (BOL), p.20

	Großer Kaisersaal	***Kleiner Kaisersaal***
15:45	IMPRS Tutor Service Sarah Körte	IMPRS Faculty Meeting
16:00	PhDNet Survey PhD representatives	
16:45	**Closing remarks** (Großer Kaisersaal) Prof. Jonathan Gershenzon, spokesperson of the IMPRS	
16:55	Talk and poster jury meets to cast votes (Großer Kaisersaal)	
17:30	Bus returns to Jena	
18:30	Café Rossini - 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

1. Home sweet home: How do desert ants know when they have arrived at their nest?
   Elisabeth Adam (HAN), p.22

2. Molecular approaches to understand carnivory syndrome in *Nepenthes*
   Alberto Davila Lara (BOL), p.23

3. Effects of the main secondary metabolites of *Physalis* plants on a specialist and a generalist species of Lepidoptera
   Pauline Sell (HEC), p.24

4. Belowground defenses in black poplar
   Sandra Lackner (GER), p.25

5. Assembling the beetle mustard-oil bomb: Glucosinolate sequestration and myrosinase activity in *Phyllotreta armoraciae*
   Theresa Sporer (HEC), p.26

6. The odour of roots: Biochemical basis of terpene biosynthesis in poplar roots
   Nathalie Lackus (GER), p.27

7. Diversity, evolutionary history and functional characterization of plant cell wall degrading enzymes in beetles of the family Cerambycidae
   Na Ra Shin (HEC), p.28

8. Convergent evolution of a metabolic switch between aphid and caterpillar resistance in cereals
   Christiane Förster (GER), p.29

9. Chlorophyll degradation in a Lepidopteran pest *Spodoptera littoralis* is mediated by a gut-specific chlorophyllide binding protein
   Vincensius Oetama (BOL), p.30

10. Volatiles released from endophytic fungi of black popular leaves
    Christin Uhe (GER), p.31

11. Revealing the role of *Plutella xylostella*'s glucosinolate sulfatase in its interactions with the specialist parasitoid *Diadegma semiclausum*
    Ruo Sun (GER), p.32

12. Two highly similar carboxy-lyases play a dual role in herbivore defense in *Populus trichocarpa*
    Jan Günther (GER), p.33

13. Could you be the One? – Identification of calcium signaling mutants
    Anja Meents (BOL), p.34

14. Evolution of conifer diterpene resin acids followed evolutionary patterns supported by the patchwork hypothesis
Andrew O'Donnell (GER), p.35

15. Modelling oscillations in biofilms
Ravindra Garde (FSU), p.36

16. Decreasing of metabolic flux in the MEP pathway during drought stress in *Picea glauca*
Erica Perreca (GER), p.37

17. Stress survival strategies of the dominating bacteria *Enterococcus mundtii* in the gut of *Spodoptera littoralis*
Tilottama Mazumdar (BOL), p.38

18. How is the terpenoid biosynthesis modified by different expression levels of isopentenyl diphosphate isomerase in transgenic gray poplar (Populus canescens) and Norway spruce (Picea abies) plants?
Toni Krause (GER), p.39

Talks

Plenary 1

Evolution of olfactory systems in Drosophilids

Lucia Prieto-Godino[1]

[1]The Francis Crick Institute
lucia.prietogodino@crick.ac.uk

Sensory systems encode the world around us to produce context-dependent appropriate behaviours. However, we know little about the way new sensory evoked behaviours arise as receptors and neural circuits are re-shaped during evolution. To bring insights into these questions, we are comparing the olfactory systems of different *Drosophila* species that have diverged in adaptation to diverse ecological niches. We found diversification both at the level of olfactory receptor proteins and neural circuit components, and identified the genetic bases behind these evolutionary changes. At the receptor protein level, through homology modelling, mutational analysis and ancient protein reconstruction, we delineated a molecular evolutionary trajectory that reveals how the specificity of a receptor has shifted multiple times from one salient odour to another. In parallel, we are using an un-biased approach to investigate the *cis*- and *trans*-regulatory changes underlying the evolutionary expansion of a neuronal population. Together, our work sheds light on important mechanisms through which neural circuits change over evolutionary time.

Plenary 2

Arbuscular mycorrhiza development and function

Caroline Gutjahr[1]

[1]Plant Genetics, School of Life Science Weihenstephan, TU Munich, 85354 Freising
caroline.gutjahr@tum.de

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

Finding a partner: Sexual communication in *Drosophila*

Mohammed A. Khallaf [1], Thomas Oliver Auer[2], Sofia Lavista Llanos[1], Dan-Dan Zhang[3], Jerrit Weißflog[1], Filip Kaftan[1], Aleš Svatoš[1], Christer Löfstedt[3], Richard Benton[2], Bill S. Hansson[1], Hany Dweck[1], Markus Knaden[1]

[1]Department of Neuroethology, Max-Planck Institute for Chemical Ecology
[2]Center for Integrative Genomics, Faculty of Biology and Medicine, University of Lausanne, Switzerland
[3]Department of Biology, Lund University, Lund, Sweden
mkhallaf@ice.mpg.de

Sexual behaviors are driven by olfaction in fruit flies. Once they land a food patch colonized by conspecifics, males almost immediately court females. *Drosophila melanogaster* generically use male-produced cis-11-octadecenyl acetate (cVA) as sex pheromone. The underlying cVA circuitry is arguably the best-studied pheromone communication system. However, little is known what regulates the social and sexual behaviors in the non cVA-producing species, like the cactophilic fly *Drosophila mojavensis*. In this study we identify previously anonymous male-specific compounds which *D. mojavensis* males transfer to females during copulation. From single molecules and genes, to neurons, to behavioral responses, we could dissect the evolution of sex pheromones perception in closely related species to *melanogaster*-species clade 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 2

Evaluation of the DREAM method for high-throughput deorphanization of chemosensory receptors

Sarah Koerte[1], Ian W. Keesey[1], Ewald Groβe-Wilde[1], Markus Knaden[1], Bill S. Hansson[1]

[1]Department of Neuroethology, Max-Planck Institute for Chemical Ecology
skoerte@ice.mpg.de

In the vinegar fly *Drosophila melanogaster* (*D. mel*) the perception of volatile compounds (olfaction) is mediated predominantly by odorant receptors (ORs), but also by gustatory receptors (GRs) and ionotropic receptors (IRs). The majority of *D. mel* ORs and IRs have been functionally characterized (deorphanized) *in vivo*, which assigned the receptors chemosignals leading to their activation and inhibition. However, most *D. mel* GRs as well as some ORs and IRs remain orphans and substantial progress in understanding the rules governing chemoperception has not been possible without ascertaining all receptors gated by a given chemosignal.
Recently it was discovered that exposure to high concentrations of odorants, above those levels found naturally, leads to decreases in the expression of certain odorant receptor genes (von der Weid *et al.* 2015). These alterations in expression can be observed after only a few hours of exposure and are reversible if the odor source is removed. Analyzing receptor expression after odorant exposure can, hence, help to identify novel receptor-ligand interactions. In this study, we evaluated the potential of the DREAM (**D**eorphanization of **r**eceptors based on **e**xpression **a**lterations in **m**RNA levels; von der Weid *et al.* 2015) technique for high-throughput deorphanization of chemosensory receptors in insect species using the model organism *D. mel*.
First, we applied the DREAM method in testing the published two receptor-ligand combinations (von der Weid et al. 2015). Using RealTime quantitative Poly-Chain-Reaction (RT qPCR) we were able to reproduce the described downregulation of target ORs. Next, we ascertained whether the DREAM technique universally qualifies as a method for the deorphanization of ORs independent of a broad or specific ligand spectrum. Here we found that only one out of six additionally tested odorant receptors showed a significant downregulation following prolonged exposure to its best known ligand. At the same time, we expanded the DREAM method to IRs, determining the applicability of the technique for chemosensory receptor classes beyond ORs. We did not observe any significant alterations in the mRNA levels of the tested IR after the odorant treatment, suggesting that the DREAM method does not work for the deorphanization of this receptor type. In single sensillum recordings (SSR) we verified the interaction of all tested odorants with their described chemosensory receptor.
In summary, we confirmed that in some cases the exposure of a receptor to high concentration of its best ligand leads to measureable downregulation. However, we did not find a universal applicability of the DREAM method for the deorphanization of all chemosensory receptors.

Talk 3

Time to learn: changing hedonic valence of an odor with experience

Florencia Campetella[1], Roman Huber[1], Markus Knaden[1], Silke Sachse[1]

[1]Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology
fcampetella@ice.mpg.de

Since the moment they are born vinegar flies need to navigate and learn about the environment around them. They need to search for food, a mating partner and a good oviposition site in which to lay their offspring. In the context of these behaviors, sensory information plays a crucial role, guiding the fly through the complex environment. For instance, when a fly encounters an odor it may approach or be repelled by it, and we may identify this behavior to be learned or innate.
During the last couple of decades we have learned quite a bit about learning and memory in the vinegar fly, and about their innate behaviors. While in the field of learning and memory, we begin to understand the mechanisms of olfactory learning and how memories are wired in the nervous system; in the field of innate behavior, studies have extensively shown and identified odor stimuli that are innately attractive or aversive to the flies, and how this distinction is made in the fly's brain. But to what extent a response is innate or learned remains unclear: are learned responses different from learned one? Are innate behaviors unable to be changed by learning? During my talk I will address these questions and present my recent finding on the topic.

Talk 4

Identification, physiological and ecological function of zwitterionic metabolites in marine diatoms

Simona Fenizia[1,2], Georg Pohnert[1,2]

[1]Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University, Jena
[2] Max Planck Institute for Chemical Ecology
sfenizia@ice.mpg.de

Diatoms are microscopically small, single-celled algae that play an immense role in keeping the planet's ecosystem functioning. They are an integral component of marine food webs and important sources of carbon, oxygen and sulfur, for higher trophic levels. Zwitterions are among the best studied algal metabolites: they are neutral natural products with both a positive and a negative charge. Their chemical ecology is highly interesting since they are able to modulate the global Sulphur cycle, to act as potential defense metabolites and as a resource for microorganisms. A large number of different zwitterionic compounds from marine microalgae were identified and their roles in the environment were pointed out. Nevertheless, according to analytical data, there are many other zwitterionic metabolites still to identify, that may exert important roles in the entire ecosystem. In this talk, I will present how LC\MS analysis allows to assign the hitherto unknown zwittermetabolome of diatoms and to discover the function of these metabolites in the environment. I will report the identification of zwitterions in three different species of diatoms, *Phaeodactylum tricornutum*, *Skeletonema costatum* and *Thalassiosira weissflogii* and the evaluation of the production, under different salinity conditions, of another important zwitterion, Ectoine, which is known to be a bacterial metabolite, but it is new in diatoms. The potential ecological and physiological functions of this metabolite will be discussed.

Talk 5

Towards FDR estimation in computational metabolomics

Martin Hoffmann[1,2], Marcus Ludwig[1], Aleš Svatoš[2], Sebastian Böcker[1]

[1]Chair of Bioinformatics, Friedrich Schiller University, Jena
[2]Max Planck Institute for Chemical Ecology
Hoffmann.m@uni-jena.de

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 a result, statistical methods for estimating the false discovery rate (FDR) of this matching process have become essential. An important prerequisite that has to be met for FDR estimation is a strong score separation between true and bogus library matches.
We show how to evaluate the score separation using Receiver operating characteristic (ROC) on the example of the current CSI:FingerID score and present two strategies to improve it.
Our results show, that calculating a p-value for the best scoring molecular fingerprint and then using that and other available data to employ machine learning, significantly improves score separation for our tool.
Following up, we use decoy databases as a tool to estimate the FDR of our method. We show how to construct and evaluate decoy databases on the basis of molecular fingerprints, and how to use them in FDR estimation.

Talk 6

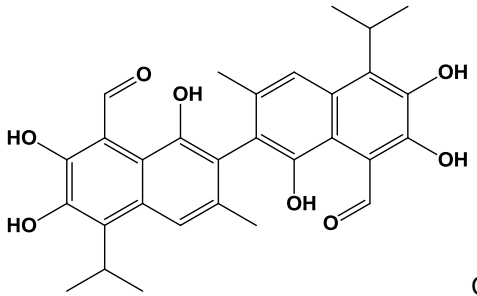
Molecular evolution and ecology of cotton secondary metabolite detoxification in Heliothine moths

Corinna Krempl[1], Nicole Joußen[1], Hanna Heidel-Fischer[1], Theresa Sporer[1], Seung-Joon Ahn[1], Michael Reichelt[2], Guillermo Hugo Jiménez-Alemán[3], Riya Christina Menezes[4], David Heckel[1], Heiko Vogel[1]

[1]Department of Entomology, Max Planck Institute for Chemical Ecology
[2] Department of Biochemistry, Max Planck Institute for Chemical Ecology
[3] Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology
[4]Research Group Mass Spectrometry/Proteomics, Max Planck Institute for Chemical Ecology
ckrempl@ice.mpg.de

Plant secondary metabolites constitute a very effective, wide-ranging, and dynamic defense system of plants and aim to negatively affect larval fitness in various ways. Especially generalist lepidopteran herbivores, feeding on many different host plants, face a wide range of diverse plant toxins and are supposed to utilize a broad spectrum of detoxification strategies. Here, we investigated the detoxification strategies of two lepidopteran generalist pest species, *Helicoverpa armigera* and *Heliothis virescens*, which allow them to feed successfully on cotton plants that produce toxic gossypol as a chemical defense compound.

First, we discovered that *H. armigera* and *H. virescens* excrete a large proportion (50%) of unmetabolized gossypol in the feces, but additionally metabolize gossypol by glycosylation. Analysis of larval feces revealed three monoglycosylated and up to five diglycosylated gossypol isomers when larvae fed on gossypol-supplemented diet. Based on their expression patterns we selected *H. armigera* candidate UGT genes and functionally expressed the respective proteins in insect cells. In enzymatic assays, we showed that UGT41B3 and UGT40D1 are capable of glycosylating gossypol mainly to a diglycosylated gossypol isomer that is characteristic for *H. armigera* and is absent in *H. virescens* feces. Second, we tested CYP6AE14, a proposed candidate enzyme for gossypol detoxification, for its ability to detoxify gossypol. In incubation assays with gossypol and heterologously expressed CYP6AE14 no metabolites were detected. Our data show that CYP6AE14 is not directly involved in gossypol metabolism, at least under the assay conditions tested, but rather takes part in the general stress response of the herbivores to plant toxins. Third, we detected high fatty acid-amino acid conjugate concentrations in larval feces after feeding on gossypol supplemented diet. We hypothesize that gossypol interferes, either directly or indirectly, with the fatty acid-amino acid metabolism, potentially resulting in negative physiological effects to Heliothine larvae. We offer novel insights into negative impacts of gossypol on larval physiology as well as the detoxification mechanism of this plant defensive toxin by two generalist herbivores.



Gossypol

Talk 7

Interaction of beetle polygalacturonases with plant cell wall proteins

Wiebke Häger[1], Roy Kirsch[1], Yannick Pauchet[1], David Heckel[1]

[1]Department of Entomology, Max Planck Institute for Chemical Ecology
wihaeger@ice.mpg.de

The herbivorous mustard leaf beetle *Phaedon cochleariae* feeds on brassicaceous plants and possesses various carbohydrases for the digestion of plant cell wall polysaccharides. Amongst those, polygalacturonases (PGs) facilitate the breakdown of the cell wall polysaccharide pectin. Several plant-derived, cell wall-associated polygalacturonase-inhibiting proteins (PGIPs) are known to inhibit microbial PGs and thus contribute to the plant´s defence against phytopathogenic fungi and bacteria. However, direct interactions between beetle PGs and plant inhibitory proteins have not yet been investigated.

Both, PGs and PGIPs, belong to multi-gene families that are believed to be shaped by an evolutionary arms race. Besides intact PGIPs, several inactive PG family members were detected in the beetle gut and PG activity was observed in the gut content. Kirsch et al. (2014) stated that “catalytically inactive proteins may act as “decoy” targets for PGIPs, thus protecting the active PGs from inhibition”.

We performed interaction studies of *P. cochleariae* PG family members (active and inactive) with crude cell wall protein extracts from the beetle’s food plant Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Putative plant PGIPs and other leucine-rich repeat (LRR) proteins were identified by MS/MS as candidates for beetle PG inhibition. Furthermore, we found differential interaction of plant proteins with the tested PG family members, indicating different specificities of plant proteins towards active and inactive PG family members. Heterologous expression of candidate proteins allows for the elucidation of protein specificity and inhibitory activity *in vitro* and will shed light on plant defence against insect PGs in general.

Talk 8

Overcome glucosinolates and radiate: How host shifts to *Brassicaceae* have led to species diversification

Matilda Gikonyo[1], Heiko Vogel[2], Franziska Beran[1]

[1]Research Group Sequestration and Detoxification in Insects, Department of Entomology, Max Planck Institute for Chemical Ecology
[2]Department of Entomology, Max Planck Institute for Chemical Ecology
mgikonyo@ice.mpg.de

Many species of the flea beetle genus *Psylliodes* (Chrysomelidae: Galerucinae) are associated with Brassicaceae plants containing the glucosinolate-myrosinase system as defense against herbivores. Upon tissue damage, glucosinolates are hydrolyzed by the enzyme myrosinase, and reactive isothiocyanates are formed that deter non-adapted herbivores. So far, it is unknown whether Brassicaceae are the ancestral host plants of *Psylliodes* beetles or whether species in this genus adapted to the glucosinolate-based defense and shifted to Brassicaceae. To answer this question we reconstruct the phylogeny of *Psylliodes* species using seven single copy nuclear genes. So far, our analysis includes 36 of the 190 described *Psylliodes* species, and these are associated with host plants in the families Brassicaceae (20 species), Solanaceae (7 species), Fagaceae (2 species), and Cannabaceae (1 species). The Brassicaceae-feeding species form the most recent clade in the current phylogeny, showing that there was a host plant shift to Brassicaceae followed by a species diversification in the new host plant range. One interesting exception is the species *P. kiesenwetteri* which also feeds on Brassicaceae, but is more closely related to species associated with Fagaceae plants. This result suggests that *Psylliodes* species adapted at least twice to the glucosinolate-myrosinase system, but how these species overcome the chemical defense in Brassicaceae is unknown. We use the cabbage stem flea beetle *P. chrysocephala*, a member of the most recent clade, as a model to elucidate this question. Our results show, that *P. chrysocephala* is not able to fully prevent the hydrolysis of glucosinolates by the plant myrosinase, and detoxifies isothiocyanates by conjugation with glutathione. Interestingly, *P. chrysocephala* can sequester and detoxify about 25% of the total amount of ingested glucosinolates, and thus evolved specific strategies to prevent the formation of isothiocyanates. The next step is to trace the molecular evolution of these strategies across the phylogeny in order to determine the key adaptation that enabled *Psylliodes* species to feed and diversify on Brassicaceae plants.

Talk 9

The sweet life: Mechanistic parallels between osmoregulation and detoxification in *Bemisia tabaci*

Michael L.A.E. Easson[1], Osnat Malka[2], Michael Reichelt[1], Christian Paetz[3], Aleksa Stanisic[1], Stephan Winter[4], Jonathan Gershenzon[1], Daniel G. Vassão[1], Shai Morin[2]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
[2]Department of Entomology, The Hebrew University of Jerusalem, Rehovot, Israel
[3]Department of Biosynthesis/NMR, Max Planck Institute for Chemical Ecology
[4]Department of Plant Viruses, Leibniz Institute DSMZ, German Collection of Microorganisms and Cell Cultures
measson@ice.mpg.de

Many piercing-sucking insects of the order Hemiptera are worldwide crop pests which can cause severe agricultural losses. While sometimes not visually obvious, the damage inflicted by these insects via phloem feeding can nevertheless cripple a healthy plant through the introduction of viruses and the deposition of sugar-rich honeydew on the leaves, leading to the growth of sooty-mould.
Plants are however not entirely without defense to this specific type of feeding, and load phloem tissue with phytoanticipins implicated in defense, for example cyanogenic glycosides and glucosinolates. In addition to those chemical defenses, phloem-feeding insects must also overcome the non-defense related challenges presented by a phloem sap-based diet, and can circumvent the extremely high osmotic potentials via biosynthesis of sugar oligomers.
Here we report the discovery of a mechanistic connection between osmoregulation and detoxification of plant chemical defenses in *Bemisia tabaci*. Our metabolic approaches identified modified plant glycosides in the honeydew of whiteflies feeding on various host plants and artificial diets containing the abovementioned phytoanticipins. Metabolites which have been modified are no longer substrates for the respective plant activating enzymes. Interestingly, it was shown that several phloem feeding insects are capable of producing these modified glycosides, indicating a general method of modification/detoxification.

IMPRS Special Talk

TReND in Africa: Bridging the gap towards a truly global scientific community

Lucia Prieto-Godino[1]

[1]The Francis Crick Institute, London
Lucia.prieto@TReNDinAfrica.org

Scientific development is pivotal for societies to innovate and solve their own problems, thus producing sustainable change. Yet, investment in science is not at the top of the agendas in most countries or non-profit organizations. Importantly, many scientists are eager to contribute towards global development, yet they find themselves not knowing how to do so, or wondering how a single individual can produce any meaningful change. In this short talk I will present the efforts we are making towards building a more global and inclusive scientific society by promoting scientific research and education in the African continent. We do so through a volunteer-based non-profit organization called TReND in Africa, which stands for Teaching and Research in Natural sciences for Development in Africa, in the talk I will explain how we got started, how it works and how you can get involved if you want.

Talk 10

Reactions of susceptible and tolerant barley genotypes after barley yellow dwarf virus infection

Maria K. Paulmann[1,2], Grit Kunert[2], Matthias R. Zimmermann[1], Nina Theis[2,3], Anatoli Ludwig[1], Doreen Meichsner[1], Ralf Oelmüller[1], Jonathan Gershenzon[2], Antje Habekuss[4], Frank Ordon[4], Alexandra C.U. Furch[1], Torsten Will[4]

[1]Department of Plant Physiology, Matthias-Schleiden-Institute for Genetics, Bioinformatics and Molecular Botany, Faculty of Biological Science, Friedrich-Schiller-University, Jena
[2]Max-Planck Institute for Chemical Ecology
[3]Biology Department, Elms College, Chicopee
[4]Julius Kuehn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Quedlinburg
mpaulmann@ice.mpg.de

Barley yellow dwarf virus (BYDV) is a phloem limited virus that is persistently transmitted by aphids. Due to huge yield losses in agriculture, the virus is of high economic relevance. Since the control of the virus itself is not possible, tolerant barley genotypes are considered as the most effective approach to avoid yield losses. Although several genes and quantitative trait loci are known and used in barley breeding for virus tolerance, little is known about molecular and physiological backgrounds of this trait. Therefore, we compared the anatomy and early defence responses of a virus susceptible to those of a virus-tolerant cultivar.

One of the very early defence responses is the transmission of electrophysiological reactions. These reactions might differ between susceptible and tolerant cultivars after infection, since BYDV causes disintegration of sieve elements in susceptible cultivars. The structure of vascular bundles, xylem vessels and sieve elements was examined using microscopy and found to be significantly decreased in size in infected susceptible plants. This could be associated with an uncontrolled ion exchange between the sieve element lumen and apoplast. Further, a potential reduced electrophysiological isolation would negatively affect the propagation of electrophysiological reactions (i.e. electropotential waves). To test the influence of BYDV infection, by leaf-tip burning induced electropotential waves (EPWs) were recorded using aphids as bioelectrodes. EPWs in infected susceptible plants disappeared already after 10 cm in contrast to those in healthy susceptible or infected tolerant or healthy tolerant plants. Another early plant defence reaction is an increase in reactive oxygen species (ROS). Using a fluorescent dye, we found a significant increase in ROS content in infected susceptible plants but not in infected tolerant plants. Similar results were found for the phytohormones abscisic acid and three jasmonates. Salicylic acid levels were generally higher after BYDV infection compared to uninfected plants. Heat stimulation caused an increase in jasmonates. By shedding light on the plant defence mechanisms against BYDV, this study, provides further knowledge for breeding viral tolerant plants.

Talk 11

TOC1 function in shoots, but not roots, mediates *Nicotiana attenuata*'s drought responses in nature

Henrique Valim[1], Erica McGale[1], Variluska Fragoso[1], Ian T. Baldwin[1] Meredith C. Schuman[1,2]

[1]Department of Molecular Ecology, Max Planck Institute for Chemical Ecology
[2]German Centre for Integrative Biodiversity Research (iDiv), Leipzig
hvalim@ice.mpg.de

The circadian clock component, TIMING OF CAB EXPRESSION 1 (TOC1), gates a plant's responses to water deficit and appears to function differently in roots and shoots. While some mechanisms responsible for *TOC1*-mediated drought responses are well understood, how *TOC1* mediates different drought responses with their corresponding fitness consequences for plants, such as drought avoidance (DA) and drought escape (DE), is poorly understood.
We micrografted roots of transgenic lines of the desert annual, *Nicotiana attenuata,* silenced in *TOC1* expression, to the shoots of empty vector (EV) control plants to investigate, in field and glasshouse experiments, the function of *TOC1* in roots, versus whole plants, in maintaining reproductive output in response to several water deficit scenarios. In *N. attenuata's* natural habitat, we quantified unripe seed capsule production under both controlled watering and ecologically realistic water deficit conditions.
While whole-plant *TOC1* silencing had severe fitness consequences for drought-stressed plants, root-specific *TOC1* silencing did not. Whole-plant *TOC1* silencing under field conditions yielded plants with lower stomatal conductance at similar leaf water potentials, suggesting stronger DA responses, while decreased conversion of biomass to seed capsules at reproductive maturity reflected limited DE responses. *TOC1* expression in shoots was sufficient to maintain high stomatal conductance under the same leaf water potential (low DA) and greater biomass-to-seed capsule conversion efficiencies (high DE) as observed in EV controls. We infer that *TOC1* function in shoots is essential for drought responses, and promotes DE rather than DA in stressed *N. attenuata* plants.

Talk 12

The role of the glucosinolate-myrosinase defense system in the interaction between *Arabidopsis thaliana* and *Sclerotinia sclerotiorum*

Jingyuan Chen[1], Daniel Giddings Vassão[1], Jonathan Gershenzon[1], Almuth Hammerbacher[1]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
jchen@ice.mpg.de

Glucosinolates (GLS) are well known plant defense chemicals in the family Brassicaceae. Hydrolysis of GLS by the enzyme myrosinase results in the production of isothiocyanates (ITCs), which were shown to be toxic to insect herbivores and fungi. The GLS-myrosinase system is activated by plant tissue disruption during insect feeding and is thought to be an important defense system against herbivores in Brassicaceae. However, it remains largely unknown how the GLS-myrosinase system influences the interaction between *Brassica* plants and pathogens. Therefore the interaction between *Arabidopsis thaliana* and an important pathogen, *Sclerotinia sclerotiorum,* was used to study the role of the GLS-myrosinase system during fungal invasion. Analysis of the major GLS hydrolysis product 4-methylsulfinylbutyl-ITC (4MSOB-ITC) in *A. thaliana* after inoculation with *S. sclerotiorum* showed that 4MSOB-ITC accumulated from 6h to 24h post inoculation. Furthermore, inoculations of wild-type *A. thaliana* Col-0 and two isothiocyanate-deficient mutants (myb28/29 and tgg1/tgg2) revealed that isothiocyanates are moderately toxic to *S. sclerotiorum* which grew slower on the WT than on the mutants. However, growth of *S. sclerotiorum* cultured *in vitro* in medium containing purified 4MSOB-ITC was not severely affected in growth, showing that, after an initial lag phase, the fungus adapted to the toxic compound. Analysis of the culture medium showed that 4MSOB-ITC was detoxified by *S. sclerotiorum* via conjugation to glutathione. Candidate genes encoding glutathione-S-transferases and ABC transporters involved in detoxification and excretion of ITCs were screened in *S. sclerotiorum* by transcript analysis of cultures incubated with different isothiocyanates. Our results suggested that the GLS-myrosinase system was activated at the initial stage of fungal infection and contributed to fungal resistance in *A. thaliana*. However, *S. sclerotiorum* also activated corresponding strategies to adapt to these plant defenses. Future research will focus on functional characterization of the candidate genes identified by transcript analysis to be involved in fungal detoxification of ITCs.

Talk 13

*Na*MPK4 plays a role in *Nicotiana attenuata's* growth responses to neighbors

Erica McGale[1], Henrique Valim[1], Deepika Mittal[1], Jesus Morales Jimenez[1], Meredith Schuman[1], Ian Baldwin[1]

[1]Department of Molecular Ecology, Max Planck Institute for Chemical Ecology
emcgale@ie.mpg.de

Mitogen-activated protein kinases (MAPKs) are part of central intracellular signal transduction cascades in all eukaryotic cells which allow organisms to respond to extracellular factors. In *Nicotiana attenuata*, mitogen-activated protein kinase 4 (MPK4) is a MAPK known to be involved in plant growth, senescence, and photosynthesis processes as well as responses to abiotic and biotic stress. Most studies on MPK4 and its orthologs in *Arabidopsis thaliana* (AtMPK4, AtMPK11, AtMPK12) and *Nicotiana tobacum* (NtMPK4) report MPK4's role in mediating a response to signals originating from herbivory or from oxidative, salinity or water stress. The role of MPK4 in growth responses to plant neighbors has not been reported. It is known that plant density typically correlates negatively with plant growth, though this is often attributed to increased competition for limited resources. In our study, we utilized a controlled watering regime to ensure the same water availability for each genotype. We found evidence that growth decreased with increased density even without direct resource competition. Paired empty-vector (EV) *N. attenuata* plants had smaller rosettes and biomasses, as well as shorter stalks than single EV plants. Interestingly, irMPK4 plants in the same experiment did not exhibit a growth response to any neighboring genotype. Our further work explores the hypothesis that MPK4 is required for neighbor-related growth responses, elaborates on the role of MPK4 in these responses, and establishes a potential function for MPK4 as a neighbor growth response mediator at the population or community level.

Talk 14

Specificity of black poplar defense responses to various insect herbivores

Thomas Fabisch[1]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
tfabisch@ice.mpg.de

Throughout the season black poplar (*Populus nigra*) trees must defend against numerous herbivore species, whose occurrence, frequency and distribution varies in time and space. Therefore spatio-temporal feeding patterns and differences in the leaf chemistry can be observed under natural conditions in the field. One important compound class taking part in black poplars chemical defense is the class of salicinoids, but also other compounds like phenolic acids, protease inhibitors and volatile organic compounds play a role in protecting *P. nigra* against insect herbivore attackers. Their underlying biosynthetic pathways are often initiated and controlled by phytohormones. Currently plant-herbivore interactions in poplar trees are intensively investigated but mostly a limited number of insect herbivore species (e.g. the generalist *Lymantria dispar* caterpillar) is tested. Only a few studies so far have investigated herbivore-species-specific patterns of poplar defense responses. In my talk I will focus on the diversity of black poplar defense responses to different herbivore attackers. I will show that salicinoids levels are less affected by herbivory and therefore show no herbivore-species-specific defense response although they show strong genotypic variation while most major groups of volatile organic compounds are induced in a herbivore-species-specific manner. I will also discuss ecological consequences resulting from this observation and highlight the importance of collecting field data additionally to laboratory experiments.

Talk 15

Finding the best pollinator by uncoupling pollinator attraction and post-pollination mate selection in *Nicotiana attenuata*

Julia Bing[1], Danny Kessler[2], Ian T. Baldwin[1]
[1] Department of Molecular Ecology, Max Planck Institute for Chemical Ecology
[2]Max Planck Institute for Chemical Ecology
jbing@ice.mpg.de

Many studies have shown that a large number of plant species prefer pollen of certain genotypes over others, i.e. mate selection. So far, most of the studies published in this field have focused on the mechanisms of mate selection after pollen deposition (post-pollination selection). An often neglected aspect in these studies is that the attraction of pollinators could be an important component of this process, since different visitors vary in their response to floral traits which the plant uses to allure potential pollinators.
In the case of *Nicotiana attenuata*, a self-compatible wild tobacco plant that grows in genetically diverse populations, the pollinator community consists of hummingbirds, hawkmoths and bees. These pollinators are known to be attracted differently by floral traits like scent and nectar. Due to huge variations in floral traits between different *N. attenuata* genotypes in a population, it is very likely that certain genotypes attract pollinators differently and thus influence what kind of conspecific pollen plants receive (pre-pollination selection). As a result, this will affect which pollen mates will be available for selection to set seeds.
In this study we combine both pre- and post-pollination selection, to investigate the importance of pollinator attraction in the process of mate choice in plants. For this we conducted natural and semi-natural experiments with transgenic lines and native accessions that differ in floral traits important for pollinator attraction. Since ethylene plays an important role in enabling the mate selection process, we used transformed plants with silenced ethylene production (ACO) which do not select mates, in comparison to empty vector control plants (EV). Pairs of EV and ACO plants were planted in a population of four native accessions that vary in floral traits such as scent emission and nectar. Seeds produced after handpollination as well as after pollinator visitation will be used for genotyping. The comparison of the seed set from both transformed lines will shed light on which pollen was brought by the pollinators (ACO) and what pollen was selected by the plant to set seeds (EV). This allows us to uncouple the events of pre-pollination selection (pollinator attraction) and post-pollination pre-zygotic

selection and therefore gives us an idea if there are pollinators that transfer exactly the pollen genotypes to the flowers that match the plant´s mate selection pattern.

Talk 16

A juvenile leaf beetle cytochrome P450 is involved in iridoid biosynthesis

Nanxia Fu[1], Maritta Kunert[1], Antje Burse[1], Wilhelm Boland[1]

[1]Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology
nfu@ice.mpg.de

Juveniles of *Phaedon cochleariae* (Chrysomelidae, Chrysomelina), a specialized herbivore, *de novo* synthesize chrysomelidial to protect themselves from predators. Previous studies indicated that early stage precursors geraniol and glucosidically bounded 8-hydrogeraniol are accumulated firstly in fat body tissue and then transferred via the hemolymph into the glandular reservoir for further conversion into the biological active iridoids.
It is hypothesized that, like its plant counterpart *Catharanthus roseus*, *P. cochleariae* recruits cytochrome P450 monooxygenases to catalyze geraniol to 8-OH-geraniol. However, whether it's the case remains exclusive. Based on combined proteomic and transcriptome analysis, we showed that *P. cochleariae* possesses 84 cytochrome P450s in the fat body tissue. Further RNAi and metabolites analysis revealed one cytochrome P450 is involved in *P. cochleariae* chrysomelidial biosynthesis. These findings broaden our knowledge on the crucial enzymes that are responsible for iridoid biosynthesis, which can be used as potential catalysts for industrial iridoid production.

Posters

Poster 1

Home, sweet home: How do desert ants know when they have arrived at their nest?

Elisabeth Adam[1], Bill Hansson[1], Silke Sachse[1], Markus Knaden[1]

[1]Department of Neuroethology, Max-Planck Institute for Chemical Ecology
eadam@ice.mpg.de

The desert ant *Cataglyphis fortis* inhabits the arid environment of the Tunisian salt pans. Foraging ants leave their inconspicuous nest entrance during the day to search for dead arthropods. Unlike other ant species from temperate regions *C. fortis* does not forage cooperatively, but performs individual foraging runs. Although these runs can cover more than 1 km, homing ants are able to pinpoint their nest with high precision using path integration as well as visual and olfactory cues.
During my PhD, I will investigate how desert ants know when they have arrived at their respective nest and how this is coded in their central nervous system. To be able to investigate those questions I will conduct behavioral experiments in the field as well as establish a desert ant colony at the lab to perform both behavioral as well as calcium-imaging experiments. This will help shed light on the question which brain regions are involved in coding nest-specific cues and in driving insect decision making.

Poster 2

Molecular approaches to understand carnivory syndrome in *Nepenthes*

Alberto Dávila-Lara[1], Heiko Vogel[2], Michael Reichelt[3], Pierre-Jean Malé[1,4], Axel Mithöfer[1]

[1]Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology
[2] Department of Entomology, Max Planck Institute for Chemical Ecology
[3] Department of Biochemistry, Max Planck Institute for Chemical Ecology
[4] Department of Organismic & Evolutionary Biology, Harvard University
adavila-lara@ice.mpg.de

The carnivory syndrome in the plant kingdom is a fascinating topic. How carnivorous plants evolved is a big question that scientists have been trying to answer for more than 150 years, and many hypotheses have been raised in this regard. Carnivory has evolved independently in five orders. This is a clear example of convergent evolution, in which different organisms manage to develop adaptations and specialize in a particular niche. These types of plants have in common to grow in often acidic environments with very low levels of nutrients, especially nitrogen. During the carnivory process, digestive enzymes degrade caught preys. These enzymes belong to PR proteins, which non-carnivorous plants use as part of plant defense against pathogens. For some similarities with non-carnivorous plant immunity, carnivory appears to have emerged from the same source. In both contexts at least three phytohormones are used as mediators. These are salicylic acid (SA), jasmonic acid (JA) or ethylene. During carnivory in *Nepenthes alata*, at least JA and SA pathways are induced. My objective is to achieve a better understanding of similarities and differences in the regulatory mechanisms within these two scenarios (carnivory vs. plant defense response). The main focus is on the regulation of the carnivory process in the presence of specific prey-derived substrates as signals and the comparison with mechanisms of defense against pathogenicity.

Poster 3

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

Marie Pauline Sell[1], Andrea Barthel[1], Yannick Pauchet[1], David Heckel[1]

[1]Department of Entomology, Max-Planck Institute for Chemical Ecology
pauline.sell@ice.mpg.de

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 4

Belowground defenses of black poplar

Sandra Lackner[1], Sybille B. Unsicker[1]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
slackner@ice.mpg.de

Upon insect herbivore attack plants induce a series of indirect and direct defenses e.g. phenolic glycosides, volatile organic compounds or protease inhibitors. When simultaneously challenged by a root feeding insect these well-known patterns become more complex. Most of the work trying to explain the dynamics of combined below- and aboveground attack was done in herbaceous plants. So far only little is known about how a woody plant species react to the combined attack of different antagonists.

While most studies focus on aboveground tissues, we investigated how belowground herbivory by cockchafer (*Melolontha melolontha*) grub, aboveground herbivory by gypsy moth caterpillars (*Lymantria dispar*) and combined attack influence the defensive chemistry of the roots of black poplar trees (*Populus nigra*).

After 4 days of belowground herbivory by *M. melolontha* grubs young black poplar trees were infested with gypsy moth caterpillars for 40 hours. Afterwards roots were harvested and defense hormones, phenolic compounds and protease inhibitors were measured.

JA and its conjugates were slightly induced after all treatments. SA was induced after aboveground herbivory and combined attack. ABA was induced after belowground herbivory and combined attack. Catechin was induced only after aboveground feeding. Protease inhibitors were strongly induced after belowground feeding but only slightly higher after combined attack compared to control levels.

The results of our study give a first impression on how trees defend their roots via secondary metabolites. In the future we will investigate other defense metabolites like polyphenoloxidases and chitinases.

Poster 5

Assembling the beetle mustard-oil bomb: Glucosinolate sequestration and myrosinase activity in *Phyllotreta armoraciae*

Theresa Sporer[1], Zhi-Ling Yang[1], Franziska Beran[1]

[1]Research Group Sequestration and Detoxification in Insects, Max Planck Institute for Chemical Ecology
tsporer@ice.mpg.de

The glucosinolate-myrosinase system (mustard-oil bomb) is the characteristic chemical defense against herbivores in crucifer plants. Upon tissue disruption, glucosinolates are hydrolyzed by the plant myrosinase to toxic isothiocyanates which repel non-adapted herbivores. We previously demonstrated that despite plant myrosinase activity, *Phyllotreta* flea beetles selectively accumulate high amounts of glucosinolates from their food plants. Remarkably, *Phyllotreta* adults also possess a beetle myrosinase and thus may use sequestered glucosinolates for their own defense. To better understand the significance of glucosinolate sequestration in *Phyllotreta*, we analyzed whether all life stages of the horseradish flea beetle *P. armoraciae* are equipped with the mustard-oil bomb. We established that both larvae and adults sequester glucosinolates from their food plant. Glucosinolates are also present in eggs and are transferred through the pupal stage to adults. Although adults feed vigorously for at least ten days after eclosion, overall glucosinolate amounts remain at comparable levels in three-, seven- and 14-days old beetles, suggesting that adults can regulate the glucosinolate concentration in their body. To test this hypothesis, glucosinolate uptake and excretion was analyzed in a feeding experiment with *Arabidopsis thaliana*. Adults accumulated new glucosinolates from *A. thaliana*, and excreted previously sequestered glucosinolates as well as glucosinolates present in *A. thaliana*. These results indicate that *P. armoraciae* adults at least partially regulate their glucosinolate level and profile by excreting sequestered glucosinolates. Interestingly, myrosinase activity differed strongly between life stages. No or low enzyme activity was detected in eggs (up to 1 day after oviposition) and pupae, respectively, possibly to prevent self-intoxication during development. On the other hand, high myrosinase activity was present in larvae (neonates and L3) and adults indicating that both larvae and adults use isothiocyanates to protect themselves from natural enemies. Our next goal is to elucidate where sequestered glucosinolates and myrosinase are stored in *P. armoraciae*, and whether *Phyllotreta* can control glucosinolate hydrolysis during feeding.

Poster 6

The odour of roots:
Biochemical basis of terpene biosynthesis in poplar roots

Nathalie Lackus[1], Sandra Lackner[1], Jonathan Gershenzon[1], Sybille B. Unsicker[1], Tobias G. Köllner[1]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
nlackus@ice.mpg.de

Plants produce and release a large variety of volatile organic compounds. These volatiles can be emitted from floral organs but also from vegetative tissues such as leaves and roots. Plant volatiles belong to several chemical classes as for instance terpenoids, green leaf volatiles, or aromatics. Vegetative volatiles are often released in response to herbivory as part of the plant defence reaction. The volatile bouquet of herbivore-treated leaves of poplar has been intensively studied. It contains a complex composition of terpenoids, which are inducible through herbivory of the gypsy moth caterpillar (*Lymantria dispar*) or the blue willow beetle (*Phratora vulgatissima)*. In comparison to the emission of above-ground volatiles, little is known about the volatiles emitted by poplar roots. In this study, we investigated the volatile blends emitted from undamaged and cockchafer larvae (*Melolontha melolontha*)-damaged roots of two poplar species: the Western balsam poplar (*Populus trichocarpa*) and the black poplar (*Populus nigra*). The volatile blends of *P. trichocarpa* as well as *P. nigra* roots were characterized by monoterpenes, independent from the treatment. Poplar contains a large family of terpene synthase genes (*tps*), which encode key enzymes of terpene biosynthesis. Two root-specific terpene synthases, PtTPS16 and PtTPS21, could be characterized and their enzymatic activity was examined *in vitro*. Although PtTPS16 and PtTPS21 were highly similar to each other, they exhibited different product specificity. While PtTPS16 produced the monoterpene γ-terpinene as major product, PtTPS21 catalyzed the formation of a monoterpene mixture dominated by camphene. A structure modeling revealed a four amino acid residue polymorphism in the active sites of PtTPS16 and PtTPS21. Using *in vitro* mutagenesis and heterologous expression of mutated proteins, we could show that three of these amino acids are key players in the product formation of the MTS.

Poster 7

Diversity, evolutionary history and functional characterization of plant cell wall degrading enzymes in beetles of the family Cerambycidae

Na Ra Shin[1], Yannick Pauchet[1]

[1]Department of Entomology, Max-Planck Institute for Chemical Ecology
nshin@ice.mpg.de

The Cerambycidae family of beetles is the most diverse group of wood-feeding (xylophagous) insects on Earth. Larvae of this family of beetles live in a challenging environment as they have evolved to consume woody tissues primarily composed of polysaccharides – the main components of the plant cell wall - recalcitrant to decomposition by producing endogenously plant cell wall degrading enzymes (PCWDEs).
Previous studies have indicated that the ability of beetles of the superfamilies Chrysomeloidea (which includes the Cerambycidae) and Curculionoidea to produce PCWDEs to break down cellulose, hemicelluloses and pectins by themselves has been acquired from microbial donors through horizontal gene transfer. However, little is known about evolutionary aspects of PCWDEs in the Cerambycidae which is a largely understudied group of insects.
To expand our knowledge on the evolutionary history and functional characteristics of PCWDEs in Cerambycid beetles, we are currently sequencing midgut transcriptomes of 22 species of this group of insects using RNA-Seq, which will be subsequently assembled them de novo. Comparative sequencing with already identified PCWDE-encoding genes will give us an idea on which of these gene families are present in the long horned beetles. Also, phylogenetic analyses will give us insights into their evolutionary history within Cerambycidae.

Poster 8

Convergent evolution of a metabolic switch between aphid and caterpillar resistance in cereals

Christiane Förster[1], Beibei Li[2], Christelle Robert[2], Tobias Züst[2], Lingfei Hu[2], Ricardo Machado[2], Vinzenz Handrick[1,4], Götz Hensel[3], Jochen Kumlehn[3], Jonathan Gershenzon[1], Matthias Erb[2]*, Tobias G. Köllner[1]*

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
[2] Institute of Plant Sciences, University of Bern, Bern, Switzerland
[3] Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben
[4] Present Address: John Innes Centre, Norwich, United Kingdom
*for correspondence: matthias.erb@ips.unibe.ch, koellner@ice.mpg.de

The capacity to tailor defense responses to different herbivores is essential for plant survival. Recent work shows that plants can use secondary metabolites with dual functions in resistance and defense signaling to mount herbivore-specific responses. To date, little is known about the prevalence, evolution and ecological consequences of this mechanism. Here, we show that *O*-methylation of DIMBOA-Glc to HDMBOA-Glc switches wheat defenses from aphid to caterpillar resistance. DIMBOA-Glc, but not HDMBOA-Glc, induces callose deposition as a defense against aphids. The induction of HDMBOA-Glc at the expense of DIMBOA-Glc following caterpillar attack increases caterpillar resistance, but decreases aphid resistance. The newly identified wheat *O*-methyltransferase gene (*TaBx10*), which converts DIMBOA-Glc to HDMBOA-Glc, is only distantly related to the respective maize genes. Thus, while the functional architecture of herbivore-specific defense regulation is similar in maize and wheat, the regulatory genes likely evolved independently from each other.

Poster 9

Chlorophyll degradation in a Lepidopteran pest *Spodoptera littoralis* is mediated by a gut-specific chlorophyllide binding protein

Vincensius Oetama[1], Stefan Pentzold[1], Yannick Pauchet[2], Wilhelm Boland[1]

[1]Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology
[2]Department of Entomology, Max Planck Institute for Chemical Ecology
voetama@ice.mpg.de

Chlorophyll is a natural pigment from plants that is synthesized and conjointly degraded in an enormous number. Chlorophyll is well studied in plants whereas there are limited findings on other organisms. As insect herbivores are organisms that naturally consume leaves and thus chlorophyll, they provide a good start to learn the degradation mechanism of Chlorophyll in non-plant organisms. Our previous studies using the notorious lepidopteran pest species, the Egyptian cotton leaf worm (*Spodoptera littoralis*) have shown that i.) Regurgitate has catalytic activity to degrade chlorophyll and ii.) Catabolites were detected inside the gut and frass (Chlorophyllide, Pheophorbide, and Pyropheophorbide). 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*. A similar gene was also found in other lepidopterans, such as *Bombyx mori* and *Helicoverpa amiverga*. First experiments on the gene expression level in different developmental stages and tissues indicate that CHBP is mainly expressed in the digestive tract in the 5th instar. Furthermore, transcript silencing via RNAinterference was applied to larvae and indicating a metabolite change as detected by LC/MS. We also found a lower survival rate in larvae injected with gene specific dsRNA, where gene expression was decreased up to 80%. Heterologous expression of CHBP in insect cells will reveal the substrate of CHBP in vitro, whereas the protein purification from regurgitate will show comprehensively the enzyme activity in each fractions. This study may have an impact in herbivore-plant interactions considering CHBP's role in survival rate, prospectively it would be of importance in agriculture to fight against lepidopteran pests.

Poster 10

Volatiles released from endophytic fungi of black poplar leaves

Christin Uhe[1], Pamela Baumann[1], Peter Biedermann[2], Sybille B. Unsicker[1]

[1] Department of Biochemistry, Max Planck Institute for Chemical Ecology
[2]Julius Maximilians University, Department of Animal Ecology and Tropical Biology (Zoology III) Würzburg
cuhe@ice.mpg.de

It is well known that plant volatiles play a major role in plant-insect interactions either as defense compounds or as attractants for insect herbivores and their natural enemies. Recently, endophytic fungi isolated from plants were also found to produce volatile organic compounds. This raises the question whether and how these fungal volatiles influence plant-insect interactions. In this study we investigate the volatile blend released *in vitro* from several endophytic fungi. The fungal species were isolated from leaves of old-growth black poplar (*Populus nigra*) trees and were identified *via* ribosomal DNA sequencing. Furthermore, we compare the fungal volatile bouquet to the volatile blend released from black poplar trees under field conditions. Fungal volatiles were collected with polydimethylsiloxane (PDMS) tubes and analyzed by GC-MS coupled to a thermodesorption unit. The volatile blends released from the different endophyte species comprise typical fungal compounds, like short-chained alcohols, as well as volatiles known from poplars. Among these the aromatic alcohol 2-phenylethanol was found in several endophytes. In addition to that, several endophytes also produce sesquiterpenes which are important components of the herbivore-induced blend of black poplar. Previous experiments have already shown that some of the fungus-derived volatiles play a role in direct and indirect plant defense. Thus we argue that the emission of volatiles from endophytic microbial species should be considered more closely in future studies investigating tree-insect interactions.

Poster 11

Revealing the role of *Plutella xylostella*'s glucosinolate sulfatase in its interactions with the specialist parasitoid *Diadegma semiclausum*

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

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
[2]Indian Institute of Science Education and Research
rsun@ice.mpg.de

Glucosinolate (GLS)-myrosinase system is the characteristic defense system of *Brassicaceae* plants against herbivorous insects. GLSs and myrosinases are stored in different compartments of leaf tissue. Herbivore's chewing ruptures these compartments, myrosinases come in contact with GLSs to catalyze their deglycosylation to produce toxic isothiocyanates (ITCs). A specialist herbivore *Plutella xylostella* has evolved a counter-adaptation glucosinolate sulfatase (GSS) which rapidly desulfatizes GLSs to form harmless desulfo-GLSs, before their deglycosylation to ITCs. To outcompete myrosinases, GSS is present in high quantity in *P. xylostella*, indicating that its possession is resource-intensive. Secondly, it has been observed that *P. xylostella* larvae feeding on hosts of different glucosinolate compositions are differentially susceptible to their natural enemies. However, whether and how *P. xylostella*'s GSS influences the natural enemy success is unknown. We propose the use of a reverse genetic method, plant-mediated RNAi by which *P. xylostella* larvae's GSSs will be silenced and the loss-of-function phenotypes will be studied *in vivo* for understanding the effect of GLSs desulfation on both herbivore and its natural enemy. In this study *Diadegma semiclausum*, a specialist hymenopteran endoparasitoid will be used as its natural enemy. This work will determine the physiological and ecological cost-benefit economics of the GSS mechanism.

Poster 12

Two highly similar carboxy-lyases play a dual role in herbivore defense in *Populus trichocarpa*

Jan Günther[1], Nathalie Lackus[1], Meret Huber[1], Axel Schmidt[1], Michael Reichelt[1], Jonathan Gershenzon[1], Tobias G. Köllner[1]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
jguenther@ice.mpg.de

Aromatic L-amino acid decarboxylases (AADCs) and aromatic aldehyde synthases (AASs) belong to the diverse group of carboxy-lyases and act on aromatic amino acids as substrate. They have been shown to play key roles in the biosynthesis of secondary plant metabolites such as feeding-deterrent amines and insect-attracting plant volatiles. Upon herbivory, the model tree species *Populus trichocarpa* accumulates phenylethylamine and emits the volatile 2-phenylethanol. The genome of *Populus trichocarpa* contains five genes with similarity to *AADC* and *AAS* genes from other plants and a RNA-Seq analysis showed that two of them were significantly expressed in caterpillar-damaged poplar leaves. Despite a high sequence similarity of about 96 %, the two genes were shown to encoded proteins with different enzymatic functions. While one enzyme (PtAADC1) catalyzed a decarboxylation of phenylalanine to phenylethylamine, the other enzyme (PtAAS1) converted phenylalanine into phenylacetaldehyde, the potential precursor for 2-phenylethanol. By in vitro-mutagenesis we showed that both enzymes are interconvertible by switching a function-dictating amino acid in the active site. *Nicotiana benthamiana* plants overexpressing *PtAAS1* emitted high amounts of 2-phenylethanol and 2-phenylethyl acetate, whereas *N. benthamiana* plants overexpressing *PtAADC1* accumulated high amounts of phenylethylamine as well as a couple of so far unidentified phenylethylamine derivatives. Interestingly, the *PtAAS1*- and *PtAADC1*-overexpressing lines accumulated 2-phenylethanol glucoside, suggesting that both enzyme products, phenylacetaldehyde and phenylethylamine, respectively, can be converted to glycosylated 2-phenylethanol *in vivo*. The concerted downregulation of *PtAAS1* and *PtAADC1* in the hybrid poplar *Populus x canescens* resulted in significantly decreased amounts of phenylethylamine and the glucosylated 2-phenylethanol, whereas levels of emitted 2-phenylethanol remained constant. Thus, we propose that PtAADC1 is responsible for the formation of phenylethylamine while PtAAS1 likely contributes to the herbivore-induced emission of 2-phenylethanol and the formation of 2-phenylethanol glucoside *in planta*.

Poster 13

Could you be the One? – Identification of calcium signaling mutants

Anja Meents[1,3], Daniel Veit[2], Wilhelm Boland[1], Ralf Oelmüller[3], Axel Mithöfer[1]

[1] Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology
[2]Max Planck Institute for Chemical Ecology Jena, Workshop
[3] Friedrich-Schiller-University Jena, Institute of General Botany and Plant Physiology
ameents@ice.mpg.de

Plants are constantly subjected to a plethora of environmental challenges. Therefore they are tailoring their responses to biotic as well as abiotic stress factors using various chemical regulators in order to transmit local information to distal tissues. One of the earliest described signaling events in plants after applying different stimuli are the changes in intracellular Calcium (Ca^{2+}) concentrations. In order to follow these calcium signatures, the non-invasive Ca^{2+} reporter aequorin serves as a suitable system to visualize local and systemically induced calcium waves in real time using a photon counting camera.

The rapid cytosolic calcium increase is known to play a pivotal role in long-range signaling within the plant, triggering local as well as systemic responses. Especially wounding by cutting the roots leads to a fast activation of molecular mechanisms throughout the plant. However, the mechanism of this wounding-specific response is not yet investigated.

Using forward genetic screening approaches after chemical mutagenesis in the model plant *Arabidopsis thalina* allows to search for a specific phenotype. This study focusses on the aequorin-based identification of a phenotype lacking a cutting-specific calcium elevation. The investigation of the molecular basis of systemic calcium signaling after wounding will be combined with establishing a standardized root-cutting method.

Poster 14

Evolution of conifer diterpene resin acids followed evolutionary patterns supported by the patchwork hypothesis

Andrew J O'Donnell[1], Bianca Fiedler[1], Jonathan Gershenzon[1], Axel Schmidt[1]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
aodonnell@ice.mpg.de

One modern question in evolutionary biology is how novel biochemical pathways arise following mutation. There is growing support that ancestral enzymes capable of binding and modifying multiple substrates can take advantage of fortuitous secondary activities once selection favors them. One supported hypothesis for this pattern of recruiting old enzymes into new pathways, known as the patchwork hypothesis, posits that new metabolic networks are formed when selection favors the novel flow and modification of metabolites between enzymes that previously performed different roles. However, details of the mechanisms by which this process occurs are lacking. For example, it is not known whether ancient enzymes that preceded duplication and recruitment into new pathways possessed the capacity to sequentially modify substrates, or if gene duplication provides the opportunity for new secondary activities to arise.

Diterpene resin acids are C_{20} tricyclic terpenoids containing a carboxylic acid and 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 examine the process of recruiting multiple enzymes into new metabolic pathways; due to the concerted catalytic activities of diTPS and CYP450 that appear to be unique to gymnosperms, biosynthesis of diterpene resin acids represents an evolutionary novelty supported by the patchwork hypothesis. The lineage of 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 further biochemical characterization of modern-day CYP720B enzymes and proposes a method for experimentally resurrecting and characterizing ancestral diTPS and CYP720B enzymes to understand the mechanisms involved in recruiting multiple enzymes together into new pathways.

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Poster 15

Modelling oscillations in biofilms

Ravindra Garde[1,2], Stefan Schuster[2], Ákos T. Kovács[3]

[1]Max Planck Institute for Chemical Ecology
[2]Chair of Bioinformatics, Friedrich Schiller University, Jena
[3]Department of Biotechnology and Biomedicine, Technical University of Denmark
rgarde@ice.mpg.de

Biofilms are an excellent example of ecological interaction among bacteria. Oscillations in biofilms are an emerging topic (Liu et. al., Nature 2015; Rotrattanadumrong et. al., Applied Physics 2017; Liu et. al., Science 2017). At the molecular level, these oscillations are due to metabolite exchange between peripheral and interior cells. With the basic model proposed by Liu et. al. we performed a computer simulation and a detailed analysis of the system. In particular, we applied the quasi-steady-state approximation to ammonia to simplify the model. We also made a comparison with the Goodwin oscillator and tried alternative rate equations to describe the activation of glutamate dehydrogenase. This theoretical analysis will provide insight into the dynamics of biofilms.

Poster 16

Decreasing of metabolic flux in the MEP pathway during drought stress in *Picea glauca*

Erica Perreca[1], Louwrance Peter Wright[2], Johann Rohwer[3], Francesco Loreto[4], Jonathan Gershenzon[1]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
[3]Department of Biochemistry, Stellenbosch University
eperreca@ice.mpg.de

The plastidial methylerythritol phosphate (MEP) pathway synthesizes non-volatile isoprenoids involved in primary metabolism, such as carotenoids and chlorophylls. Furthermore the MEP pathway produces isoprene, the most emitted biogenic volatile compound on earth, assumed to have a defense role against abiotic stresses. The conifer species *Picea glauca* is the most common spread in the Boreal forest supposed to be more subjected to the drought stress episodes in the frame of the climate change. We investigate how does drought stress affect the regulation of the MEP pathway and change the production of isoprene and non-volatile isoprenoids. We measured the metabolic flux in the pathway by using the ^{13}C labelling and mathematical modelling during different levels of drought stress. We found a decrease in the metabolic flux during a moderate drought stress level, which probably led a slight reduction of carotenoids and chlorophylls, whereas the isoprene emission decreased only during severe drought stress conditions. These results suggest an important role of isoprene during drought stress.

Poster 17

Stress survival strategies of the dominating bacteria *Enterococcus mundtii* in the gut of *Spodoptera littoralis*

Tilottama Mazumdar[1], Beng Soon Teh[1], Wilhelm Boland[1]

[1]Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology
tmazumdar@ice.mpg.de

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 5th 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?

A GFP-tagged reporter *E. mundtii* has been constructed to answer our questions. They are fed to the insects at early instars, and sorted from the gut spatially and temporally using flow-cytometry. A simultaneous transcriptomic analysis of the retrieved bacteria and the host gut tissue must tell us how they interact.

The fluorescent reporter confirmed the persistence of *E. mundtii* in the gut. Also, RNA-sequencing of the sorted bacteria has given us preliminary answers to some questions. There are upregulated pathways for stress survival: alkaline stress, biofilm formation, two-component signaling systems, resistance towards oxidative stress. There is a differential regulation among various metabolic pathways too.

Further validation, plus sequencing the host transcriptome will give us the full picture of their interaction.

Poster 18

How is the terpenoid biosynthesis modified by different expression levels of isopentenyl diphosphate isomerase in transgenic gray poplar (*Populus canescens*) and Norway spruce (*Picea abies*) plants?

Toni Krause[1], Maricel Santoro[1], Erica Perreca[1], Jonathan Gershenzon[1], Axel Schmidt[1]

[1]Department of Biochemistry, Max Planck Institute for Chemical Ecology
tkrause@ice.mpg.de

Isoprenoids are the most functionally and structurally diverse group of plant metabolites with a large variety of functions and originate from the head-to-tail condensation reaction of the five-carbon unit isopentenyl diphosphate (IDP) and its allylic isomer dimethylallyl diphosphate (DMADP). One of the key enzymes in the biosynthetic pathway is the isopentenyl diphosphate isomerase (IDI) catalyzing the isomerization between IDP and DMADP.

But isoprenoid biosynthesis varies among plants. Woody plants from the Salicaceae and Pinaceae are known to emit additionally the hemiterpene isoprene that is produced from DMADP, which makes the regulation of isoprenoid biosynthesis more complex than in non-isoprene emitting plants.

To get more insights into the regulation of isoprenoid biosynthesis, we will compare a species that emits high quantities of isoprene and low levels of other isoprenoid secondary metabolites, the gray poplar (*Populus canescens*), with Norway spruce (*Picea abies*), a species making only low amounts of isoprene but large quantities of terpene oleoresins containing mono- and diterpenes. To alter isoprene emission, IDI has been targeted in a transgenic approach to generate plants that show either overexpressed or silenced IDI expression levels.

Transgenic poplar interestingly shows a reduction in isoprene emission of 56% and 25% in RNAi-plants with reduced IDI-expression levels and plants overexpressing IDI, respectively. Mono- and Sesquiterpenoid emission was not affected in the transgenic lines, but RNAi plants showed strong accumulation of isoprenyl acetate (3-Methyl-3-Buten-1yl acetate), which was present 500 times more than in control plants and might be a degradation product of IDP. The detection of this compound leads to the assumption, that poplar possesses another yet unknown regulatory mechanism to stabilize the intracellular equilibrium of IDP and DMADP. Additionally, the degradation of IDP to isoprenyl acetate might involve an acetyl transferase with broad product specificity similar to the Populus trichocarpa benzoyl-CoA:salicyl alcohol O-benzoyltransferase, which was significantly upregulated in RNAi plants.

In the next step, the transgenic lines will be characterized regarding other isoprenoid metabolites and the internal pools of IDP and DMADP, to get a further understanding of how isoprenoid biosynthesis is regulated.

By a detailed characterization of the transgenic plants, we hope to contribute to a better understanding of how IDI, isoprene emission and isoprenoid production are linked within the plant's biosynthetic machinery.