



**IMPRS**

Exploration of Ecological  
Interactions with Molecular  
and Chemical Techniques



# 14<sup>th</sup> IMPRS Symposium

February 25-26, 2015

Altes Schloss, Dornburg



# Program

## Wednesday, February 25, 2015

- 08:00      *Departure, central bus stop (opposite Jena Paradies train station)*
- 08:45      **Welcome and Announcements**  
Prof. Jonathan Gershenzon, spokesperson of the IMPRS
- 09:00      **Plenary Lecture:**  
Prof. Giovanni Galizia, Universität Konstanz  
Odor coding, identification and evaluation in insect neural networks  
  
Chair: Roman Huber
- 10:00      *Coffee break*
- 1st session, Insect olfaction and herbivory**  
Chair: Sarah Körte
- 10:20      Preference of *Drosophila melanogaster* larvae toward different fruits  
Ebrahim, Shima
- 10:40      cAMP imaging in *Drosophila melanogaster* olfactory sensory neurons  
Miazzi, Fabio
- 11:00      Exploring the RNAi core machinery in Lepidoptera species  
Neunemann, David
- 11:20      Cellulose-digestion in herbivorous beetles  
Busch, Andre
- 11:40      *Manduca sexta's*  $\beta$ -glucosidase mediated unusual counter-defense against its host's most abundant chemical defenses, the diterpene glycosides  
Poreddy, Spoorthi
- 12:00      Intraspecific diversity in plant jasmonate signaling alters plant competitive outcomes and herbivore damage by three native herbivores  
Adam, Nora
- 12:20      *Lunch*
- 2nd session, Plant-herbivore interactions**  
Chair: Xiang Li
- 13:20      Cytokinin-mediated regulation of plant development controls herbivore resistance in *Nicotiana attenuata*  
Brütting, Christoph
- 13:40      Effects of glucosinolates and isothiocyanates on the development, metabolism and chemistry of *Spodoptera littoralis*  
Jeschke, Verena
- 14:00      Timing for indirect defense; circadian rhythm in biosynthesis of green leaf volatiles determines timing of predator attraction  
Joo, Youngsung
- 14:20      Jasmonate-dependent depletion of plant carbohydrates constrains resistance and

tolerance against herbivores  
Ruiz Machado, Ricardo

14:40 Insect herbivore elicits genome-wide alternative splicing responses in *Nicotiana attenuata*  
Ling, Zhihao

15:00 Coffee Break

15:50 **Poster Talks I – odd numbers (1 slide and 1 min/poster)**  
Chair: Nanxia Fu

16:00 **Poster Session I – odd numbers**

17:30 *End of poster session*

17:45 *Return to Jena by bus (central bus station)*

## Thursday, February 26, 2015

- 8:00 *Departure, central bus stop (opposite Jena Paradies train station)*
- 8:45 **Plenary Lecture:**  
Prof. Dorothee Staiger, Universität Bielefeld  
RNA-binding proteins in biological timing and pathogen defense  
  
Chair: Franziska Eberl
- 09:45 *Coffee break*
- 3rd session – Carnivory, insect chemistry and plant defense**  
Chair: Jingyuan Chen
- 10:10 Regulation of carnivory-related signaling in *Nepenthes*  
Yilamujiang, Ayufu
- 10:30 The dark matters: A belowground herbivore drives the evolution of root secondary metabolites in nature  
Huber, Meret
- 10:50 Through the looking-glass, and what *Spodoptera* found there – detoxification of maize chemical defenses  
Christoff Wouters, Felipe
- 11:10 Legume chemistry and the specificity of pea-aphid host races  
Sanchez Arcos, Carlos
- 11:30 Harmonine – the defense alkaloid of the Asian lady beetle *Harmonia axyridis*  
Nagel, Nadja
- 11:50 *Spodoptera littoralis* detoxifies neurotoxin 3-nitropropanoic acid through conjugation with amino acids  
Novoselov, Alexey
- 12:10 *Lunch*
- 4th session: Bacterial interactions**  
Chair: Theresa Hölscher
- 13:10 You are what you eat – Can selective advantages explain the AT-bias of endosymbiotic genomes?  
Dietel, Anne-Kathrin
- 13:30 The bacterial network: Nutrient exchange via nanotubes  
Shitut, Shraddha
- 13:50 Timing is everything: transcription speed and stress response in *Escherichia coli*  
Großmann, Peter
- 14:10 Geographical stability of an ecologically important gut microbiota pine weevils  
Berasategui, Aileen
- 14:30 Correcting mass errors: Computational recalibration of mass spectrometry imaging data  
Kulkarni, Purva
- 14:50 Native root-associated bacteria protect their host plant from a fungal sudden-wilt disease via induced systematic resistance and allelopathy  
Rakesh Santhanam

- 15:10 *Coffee Break* + IMPRS student meeting
- 15:50 **Poster Talks II – even numbers (1 slide and 1 min/poster)**  
Chair: Ahmed Mohamed
- 16:00 **Poster Session II – even numbers**
- 17:30 **Final Remarks**  
Prof. Ralf Oelmüller, spokesperson of the IMPRS
- 17:45 *Return to Jena by bus*
- 18:15 *Discussion of quality of posters and talks between the students and guest speakers, meeting at Café Fly*

## Poster presentations

- 1) Confusion by the background: Try to smell your sex partner!  
Badeke, Elisa
- 2) Laser-assisted methods for spatially-resolved metabolomics  
Bartels, Benjamin
- 3) A pollen's journey: Which pollinator offers the best lift?  
Bing, Julia
- 4) The roles of gene duplication in the evolution of anti-herbivore defenses in *Nicotiana attenuata*  
Brockmüller, Thomas
- 5) Chemical Defense responses of *Arabidopsis thaliana* to infection by *Sclerotinia sclerotiorum*  
Chen, Jingyuan
- 6) Poplar responses to simultaneous herbivore and pathogen attack  
Eberl, Franziska
- 7) Unravelling 2-deoxy-2-fluoro-D-glucose metabolism in plant tissue using mass spectrometry and NMR  
Fatangare, Amol
- 8) The absolute configuration of salicortin, HCH-salicortin and tremulacin from *Populus trichocarpa x deltoids* Beaupré  
Feistel, Felix
- 9) Crystallization and Structure analysis of a *Phaedon cochleariae* Isopropyl diphosphate synthase (*PciDS1*)  
Fu, Nanxia
- 10) Different biosynthetic routes leading to the formation of 2-phenylethanol in *Populus trichocarpa*  
Günther, Jan
- 11) Benzoxazinoids: Biosynthesis and function of major defense compounds in maize  
Handrick, Vinzenz
- 12) Smelling a low-cost meal: Hawkmoth use olfactory information to optimize their foraging behavior  
Haverkamp, Alexander
- 13) CML9 – A calcium sensor protein involved in plant defense?  
Heyer, Monika
- 14) Genetic background, ploidy and ovule-directed transcriptome analysis: a biogeographic approach to understanding apomixis penetrance in *Poa pratensis*  
Hilpert, Stephanie
- 15) Navigation during long foraging paths of desert ants  
Huber, Roman
- 16) Partners in Olfaction: the influence of SNMPs and OBPs on olfactory signaling in *Drosophila*  
Körte, Sarah
- 17) Small Molecule Signals in the soil dwelling nematode *Oscheius tipulae*  
Kuhlisch, Cornelius

- 18) Extraction of stylar fluid after controlled mixed pollinations in *Nicotiana attenuata* and subsequent *in vitro* pollen tube growth bioassay  
Li, Xiang
- 19) Think before making a decision  
Mohamed, Ahmed
- 20) To go or not to go? Behavioral responses to binary mixtures of attractive and aversive odors  
Retzke, Tom
- 21) Glucosinolate detoxification and sequestration – two beetles, two pathways  
Shekov, Anton
- 22) Carbon Isotope Ratios Determination: Applying *in vitro* enzyme-catalytic model to *in vivo* study  
Tan, Wenhua
- 23) Elucidating the molecular mechanisms in a defensive beewolf-*Streptomyces* symbiosis  
Tang, Eric
- 24) Social networking in the underground - an ectomycorrhizal story  
Wagner, Katharina

# Talks



## **Preference of *Drosophila melanogaster* larvae toward different fruits**

Shimaa A. M. Ebrahim, Hany K. M. Dweck, Bill S. Hansson, Markus Knaden

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The vinegar fly *Drosophila melanogaster*, which utilizes fermenting fruit as breeding a substrate, likewise assesses a wide range of factors prior to choosing its oviposition sites. The capacity to discriminate and choose appropriate sites for oviposition is of profound importance to the fitness of the future generation. Adult Flies prefer citrus fruits as oviposition substrate. This preference mediated via or19a receptor. Interestingly, or19a is absent in the larvae. We asked whether the *Drosophila* larvae exhibit a preference toward certain fruit. Our results show passion fruit and pineapple were the most attractive fruit in multiple choice and two choice assays. We found the preference of *Drosophila* larvae is reflected in the olfactory system. In survival rate, mango was the suitable fruit. On the other hand mandarin showed the shortest life cycle. While the longest life cycle recorded on plum and apple. These results indicate that the adult and larvae have different preference from each other.

## **cAMP imaging in *Drosophila melanogaster* olfactory sensory neurons**

Fabio Miazzi, Sabine Kaltofen, Dieter Wicher, Bill S. Hansson

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Insects smell using two pairs of olfactory organs, the antennae and the maxillary palps, located in the anterior part of their head. These organs are covered with hair-like structures called sensilla, which house the dendrites of olfactory sensory neurons, carrying the olfactory receptors. Insects possess three types of olfactory receptors: Ionotropic Receptors (IRs), Gustatory Receptors (GRs) and Odorant Receptors (ORs). ORs, in particular, are involved in the perception of a plethora of behaviourally relevant organic compounds, like food odours, danger odours (e.g. Geosmin) and pheromones. Despite significant progress in the knowledge of the structure of ORs, the signal cascade that leads to olfactory perception and its regulation is still controversial.

We recently established a new preparation that allows us to expose olfactory neurons in *Drosophila* antennae. This technique makes possible to stimulate these cells and to monitor the response of individual olfactory neurons expressing genetically encoded fluorescence indicators using functional imaging techniques. Moreover, coupling imaging analysis with pharmacological experiments it is possible to study olfactory transduction in *ex vivo* conditions.

We applied this technique to perform cAMP imaging following odour stimulation, using the Epac1-camps cAMP indicator. Here we show preliminary data suggesting that insect ORs activation leads to cAMP production, showing for the first time a direct proof that ORs are not only ionotropic, but also metabotropic channels.

## Exploring the RNAi core machinery in Lepidoptera species

David Neunemann, Heiko Vogel and David Heckel,

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Gene silencing via dsRNA has become a powerful tool to explore functional genomics in a wide variety of eukaryotic organisms. However, RNAi is still in the exploratory phase in non-model organisms. So far, several potentially limiting factors for RNAi in Lepidoptera have only been proposed for *Bombyx mori*. In the past years, RNAi in Lepidoptera was shown to be not as straight-forward as in other insect species, and that its establishment is not trivial due to highly variable efficiencies of gene knock-down experiments.

To get an idea why RNAi in Lepidoptera is difficult to establish, I annotated genes which are known to be involved in the miRNA- and siRNA-pathway, with the help of our genome sequence databases of *Helicoverpa armigera* (*Ha*) and *Heliothis virescens* (*Hv*).

For the miRNA pathway I focus on Dicer-1, Argonaute-1, Loquacious, Drosha and Pasha. Additionally, I was looking for Dicer-2, Argonaute-2 and R2D2 as siRNA pathway target genes and I am also looking for distinct auxiliary factors like Translin and SID-1 which are necessary for dsRNA spreading and dsRNA transport, respectively. I analyzed these gene transcripts via qRT-PCR in several tissues of 5<sup>th</sup> instar larvae.

Compared to all other RNAi-related genes, R2D2 is transcribed at very low levels in all tissues except testes, whereas Loquacious (similar to R2D2 in the siRNA pathway) is transcribed at very high levels in all tissues. These results, in combination with our analysis of RNAi experiments in Lepidoptera, could suggest that, despite appropriate design, the dsRNA failed to enter the siRNA pathway, and to knock-down the gene of interest, due to the observed very low levels of R2D2.

The siRNA pathway is also known as the “antiviral pathway” and defends the organism against RNA and DNA viruses. Based on our initial results, I want to find out if it is possible to activate the siRNA pathway (and thus R2D2 expression) with a Baculovirus. To test that I infected larva with an *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). 48h post infection I could measure an increase of R2D2 expression in the gut and rest body samples.

In another project I will try to downregulate genes in the testes, because all RNAi core genes are expressed there. A first approach will be to test the uptake of dsRNA into the testis with TM-Rhodamine labeled dsRNA.

## Cellulose-digestion in herbivorous beetles

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Cellulose is a major component of the plant primary and secondary cell wall consisting solely of glucose moieties linked together through  $\beta(1\rightarrow4)$  glycosidic bonds. Due to the domination of plants on our planet, cellulose is also considered as the most abundant biopolymer on earth, thus making it a generous metabolic energy source. Having this in view, it is striking that cellulose degradation is scarce and mostly restricted to cellulolytic microorganisms. Acting as symbionts these microorganisms also allow animals (e.g. Termites) to digest cellulose. However, in recent years various metazoans have been discovered that encode their own set of cellulases giving them the ability to degrade cellulose independently of cellulolytic symbionts. In our previous research, genomes of beetles of the Chrysomeloidea (leaf beetles and longhorned beetles) and the Curculionoidea (weevils and bark beetles) superfamily were found to encode putative cellulases belonging to the glycoside hydrolase family 45 (GH45). Of the six beetle species I am investigating the number of genes encoding putative GH45 cellulases varies from two transcripts to up to eleven. The following questions arise:

Are they all cellulases or have evolved other functions? Have they evolved from an ancestral gene or were they acquired by horizontal gene transfer (HGT). If so, from what organism were they acquired from? How many genes were acquired? When did the HGT take place? Why does the number of GH45 genes between different beetle species differ so much?

By the means of PCR-based cloning, heterologous expression and subsequent characterization assays on agarose plates containing carboxymethyl cellulose (CMC) and thin layer chromatography, I was able to identify 13 active cellulases out of a total of 34 GH45 proteins. At least one GH45 per species showed activity on CMC. The remaining 21 GH45s show neither activity on CMC nor on any other substrate tested (xylan, xyloglucan or galactomannan). Supported by our phylogenetic analysis, we believe that the GH45s have evolved from two ancestral GH45s present in the last common ancestor of the Curculionoidea and Chrysomeloidea. Currently, I am investigating the enzymatic properties of GH45 cellulases in more detail. Also under investigation is whether GH45s in beetles were acquired by HGT. In the near future I am going to conduct RNAi experiments followed up by phenotype observation to get first insights into the potential role of inactive GH45s.

## **Manduca sexta's $\beta$ -glucosidase mediated unusual counter-defense against its host's most abundant chemical defenses, the diterpene glycosides**

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*Nicotiana attenuata* produces 17-hydroxygeranylinalool diterpene glycosides (HGL-DTGs) in starch-equivalent concentrations, against its specialist lepidopteran herbivore *Manduca sexta*. Lyciumoside IV and its malonylated forms, nicotianoside I and II, constitute ~80% of these HGL-DTGs. We discovered the counter-defense mechanism of *M. sexta* against HGL-DTGs using lyciumoside IV and its malonylated forms as model HGL-DTGs. Upon ingestion, nicotianoside I and II are rapidly and non-enzymatically demalonylated to lyciumoside IV, by the alkalinity of larval oral secretion. Then lyciumoside IV is detoxified in the midgut to form a novel compound, 3-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosyl-17-hydroxygeranylinalool (RGHGL) by  $\beta$ -glucosidase 1 catalyzed deglycosylation. This type of detoxification by deglycosylation is unique because typically, the deglycosylation of glycosylated phytochemicals by insects results in the opposite: toxin activation. We used a reverse genetics approach, plant-mediated RNA interference (PMRi) to silence *M. sexta*'s  $\beta$ -glucosidase 1. The  $\beta$ -glucosidase 1-silenced larvae were impaired in lyciumoside IV deglycosylation and showed molting impairments and higher mortality. To examine the consequences of this detoxification on tritrophic interactions, we planted  $\beta$ -glucosidase 1-silencing PMRi plants into the hostplant's native habitat. A native predatory-spider *Camptocosa parallela* captured and killed control and  $\beta$ -glucosidase 1-silenced larvae at similar frequencies but ingested only 25% of  $\beta$ -glucosidase 1-silenced larvae; ingestion resulted in locomotor distress for the spiders. While spiders equally attacked and ingested RGHGL-coated or -ingested larvae, they were deterred by the lyciumoside IV-coated larvae. Although lyciumoside IV deters spiders, it is not defensively co-opted by *M. sexta* to avoid its deleterious effects such as molting impairments and mortality. This study reveals the importance of oral secretions in xenobiotic metabolism and demonstrates that deglycosylation is an effective detoxification strategy for insects, as it is for fungi.

## **Intraspecific diversity in plant jasmonate signaling alters plant competitive outcomes and herbivore damage by three native herbivores**

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*Nicotiana attenuata* plants in natural populations differ in their jasmonate (JA) defense hormone biosynthesis, signaling, and associated defense metabolites. Variation among individuals in stress signaling systems might increase populations' productivity and resistance under biotic stress, such as attack by multiple herbivore species. In this study, we showed that populations of the wild tobacco *Nicotiana attenuata* comprising two genotypes differing in their expression of a single JA biosynthetic gene (*LOX3*, *Lipoxygenase 3*) have higher productivity than monocultures of either genotype when populations are challenged with three native herbivores. This results from a combination of differences in plant competitive ability, which are inversely correlated to JA production; and plant-mediated negative interspecific interactions among the herbivores. We analyzed this complex interaction among three native herbivores and populations of their host plants in a mesocosm experiment and addressed plant growth and fitness consequences at individual and population levels. All populations were challenged sequentially with the generalist leafhopper *Empoasca* sp., the specialist mirid *T. notatus*, and specialist caterpillar *Manduca sexta*. *T. notatus* avoids plants infested with *Empoasca* sp., which in turn feeds preferentially on individual plants with reduced JA accumulation. However in populations having equal numbers of wild-type plants and asLOX3 plants rendered JA-deficient by RNAi, *Empoasca* sp. feeding is distributed among both JA-producing and JA-deficient genotypes, and this, rather than plant JA production, determines damage patterns from *T. notatus*. Finally, feeding damage and growth of voracious *M. sexta* larvae also differs in mixed versus monocultures. Thus in the end, WT plants growing in polycultures produced more flowers and seed than WT plants growing in monocultures, whereas the flower and seed production of asLOX3 plants in polycultures did not differ in comparison to those growing in monocultures.

## **Cytokinin-mediated regulation of plant development controls herbivore resistance in *Nicotiana attenuata***

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Plant defense pathways that mediate resistance against herbivores are well described. Whether plant development and its associated regulation of growth hormones controls herbivore resistance has not been tested rigorously. Here we analyzed the role of cytokinins (CK) in ontogeny-dependent herbivore resistance in the annual plant *Nicotiana attenuata*.

Using novel transgenic tools in *N. attenuata* we were able to uncouple CK levels from its developmental regulation. We systematically analyzed the resistance of juvenile and reproductive-stage *N. attenuata* plants to larvae of the specialist herbivore *Manduca sexta* and tested the role of CKs in regulating age-dependent resistance to herbivores.

We found a negative correlation between plant or leaf age and *M. sexta* performance, which is consistent with the activation of senescence-related processes by *M. sexta* feeding in reproductive-stage plants. While decrease in larval mass gain in older plants cannot be explained by levels of defensive metabolites or carbohydrates, we found a positive correlation with protein and amino acid contents. Herbivore attack increased defense metabolites in young plants but decreased levels of nutrients in flowering plants. Uncoupling CK levels from plant development or regulation by herbivore attack and inhibiting senescence processes with transgenic plants increased protein and amino acid contents in attacked leaves and performance of specialist herbivores. As the higher mass gain of *M. sexta* in transgenic plants with higher CK levels occurred regardless of simultaneously increased levels of defense compounds, we hypothesize that herbivore triggered senescence-related processes are key components of a plants anti-herbivore strategy that might be worth focusing on.

These data establish a link between plant development and herbivore resistance and demonstrate that senescence-related processes and their regulation by CKs are integral components of plant responses to herbivore attack. Our findings are discussed in the context of the optimal defense theory.

## Effects of glucosinolates and isothiocyanates on the development, metabolism and chemistry of *Spodoptera littoralis*

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Some small insect herbivores feed successfully on plants containing glucosinolates, despite their diverse array of harmful and deterrent breakdown products. Isothiocyanates (ITCs) are considered to be the most toxic glucosinolate-derived metabolites and are typically thought to be responsible for the reduced growth and delayed development observed in several generalist insects feeding on glucosinolate-producing plants. Some insects detoxify ITCs via conjugation with glutathione (GSH), but a large amount of ITCs remains unmodified and may lead to the observed ill-effects in the insects. We compared the effects of aliphatic and indolic glucosinolates, which form distinct ITC and non-ITC hydrolysis products, against two generalist chewing herbivores in a lab set-up. The development of larvae of *Spodoptera littoralis* (African cotton leafworm) was investigated from hatching until adult emergence while the larvae were reared on *Arabidopsis thaliana* Col-0 and the glucosinolate-deficient mutants *myb28myb29* (deficient in aliphatic glucosinolates), *cyp79B2cyp79B3* (deficient in indolic glucosinolates), and *myb28myb29cyp79B2cyp79B3* (deficient in both aliphatic and indolic glucosinolates). Comparing growth and instar durations, we found that both types of glucosinolates alone negatively affect larval development, but in combination their effect is significantly stronger. To our surprise, the negative effects of the glucosinolates on insect weights were inverted for pupae and adults.

Furthermore, in order to understand the mechanisms of ITC toxicity, we determined how these compounds disturb the biochemistry and metabolism of *S. littoralis*. We investigated changes in physiological processes and chemistry of different body tissues after feeding the aliphatic 4-methylsulfanylbutyl-ITC (sulforaphane) in an artificial diet. The most typical effect is the decrease of GSH in the midgut tissue and hemolymph, likely due to losses by conjugation to ITC during detoxification. As a consequence, the levels of free amino acids are altered, in particular that of cysteine. Secondly, a characteristic symptom of ITC intoxication is a reduction in protein levels in the integument. As a result, the proportion of fat increases correspondingly. In combination, these effects contributed to the reduced performance of generalist insect herbivores feeding on glucosinolate-/isothiocyanate-containing diets. Further studies employing transcriptomic and proteomic techniques will be performed.



## Timing for indirect defense; circadian rhythm in biosynthesis of green leaf volatiles determines timing of predator attraction

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Plants emit fatty acid-derived green leaf volatiles (GLVs) when damaged by mechanical wounding or herbivore attack. Although GLVs are immediately released by damage, there is also fast metabolism of GLV aldehydes to alcohols and esters upon wounding. We found that the amount and composition of GLVs emitted by herbivore-induced *Nicotiana attenuata* plants differed over the course of a day. Morning blends have lower total amounts of GLVs but a higher ratio of esters to alcohols and aldehydes, and a morning blend was more attractive to day predators than an evening blend. To test how the diurnal fluctuation of GLVs is generated in *N. attenuata*, we measure the transcript of the biosynthetic enzymes *LIPOXIGENASE2 (LOX2)*, *HYDROPEROXIDE LYASE (HPL)*, and *ALCOHOL DEHYDROGENASE (ADH)*. All biosynthetic genes showed a nocturnal rhythm in *N. attenuata* leaves under light/dark conditions, and *HPL* transcripts maintained their rhythm under constant light conditions. Furthermore, silencing *LATE ELONGATED HYPOCOTYL (LHY)*, a core component in the plant circadian clock, in *N. attenuata* shifted the peaking time of *HPL* transcript expression and altered the abundance of GLV emission under natural conditions. Circadian regulation of *NaHPL* suggested that the circadian clock regulates GLVs biosynthesis. To separate biosynthesis from emission, we furthermore measured concentrations of GLVs from intact leaves of *N. attenuata*. Interestingly, the fluctuations of GLV levels in leaves were maintained under constant light, diurnal, and natural conditions, and thus follow the plant endogenous rhythm. This rhythm was altered in *LHY*-deficient plants. Taken together, these results indicate that diurnal patterns of GLVs emission play important roles in plant indirect defense and the plant circadian clock regulates timely biosynthesis of GLVs in leaves.

## Jasmonate-dependent depletion of plant carbohydrates constrains resistance and tolerance against herbivores

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While the behavior and function of plant secondary metabolites in herbivore-attacked plants is well studied, less is known about primary metabolites in this context. We found that *Manduca sexta* attack dramatically decreased carbohydrates, including glucose, fructose, sucrose and starch, in *Nicotiana attenuata* leaves and roots. This effect was not observed in jasmonate-deficient transgenic plants, suggesting that carbohydrate depletion is jasmonate-dependent [1, 2]. The reduction in sugars and starch may have two consequences for the plant: First, it may change its nutritional value for herbivores and thereby affect herbivore resistance. Second, it may reduce the capacity of non-attacked tissues, including for instance the roots, to supply energy for regrowth following herbivore attack. We addressed these two questions in detail in two separate sets of experiments [1, 2]. To understand the impact of carbohydrate depletion on herbivore resistance, we manipulated leaf carbohydrates through genetic engineering and *in vitro* complementation. Contrary to our expectation, both *in planta* and *in vitro* approaches showed that the lower sugar concentrations led to increased *M. sexta* growth, suggesting that carbohydrate depletion constrains rather than enhances herbivore resistance. To understand the impact of carbohydrate depletion on herbivore tolerance, we combined natural variation and genetic manipulation of jasmonate signaling and carbohydrate allocation and measured their regrowth capacity upon herbivore attack. The results suggest that the herbivory-induced, jasmonate-dependent depletion of root carbohydrates significantly constrains the plant's regrowth capacity and fitness upon insect attack. Taken together, these experiments demonstrate that jasmonate-dependent carbohydrate depletion reduces both resistance and tolerance to foliar herbivory.

1. Machado, R.A.R., et al., Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. *New Phytologist*, 2013. 200(4): p. 1234-1246.
2. Machado, R.A.R., et al., Jasmonate-dependent depletion of soluble sugars compromises plant resistance to *Manduca sexta*. *New Phytologist* (in press), 2015.

## **Insect herbivore elicits genome-wide alternative splicing responses in *Nicotiana attenuata***

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The changes in gene expression and alternative splicing (AS) regulate many responses to abiotic and biotic stresses in eukaryotic organisms. Using *Nicotiana attenuata*, an ecological model plant with available draft genome as study system, we specifically ask 1) whether insect herbivore can elicit AS responses in plants. 2) if yes, what are the regulation mechanisms? By using mRNA sequencing, we performed a genome-wide analysis on *Manduca sexta* feeding induced AS in both leaves and roots of *N. attenuata*. We found *M. sexta* feeding can induce AS responses in both plant tissues with great effects in roots than leaves. The induced AS responses were precisely regulated and likely contribute to the transcriptomic fine tuning and anti-herbivore defenses. In addition, the strong AS responses elicited in *N. attenuata* roots by *M. sexta* feeding on leaves were likely due to the up-regulation of several serine/arginine-rich (SR) and SR-like genes that were regulated through JA dependent manner. Lastly, the *M. sexta* feeding induced regulation of AS and gene expression were independent from each other in both tissues. In future, we will employ a comparative approach to investigate the evolution of genome-wide AS conservation and changes among different plant species.

## Regulation of carnivory-related signaling in *Nepenthes*

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Carnivory in plants is an adaptation to nutrient-poor environments. Carnivorous plants obtain additional mineral nutrients by trapping and digesting (insect)-prey with specialized organs. Those organs include pitchers, metamorphosed leaves, containing a digestive fluid. Pitchers are employed by species of the genus *Nepenthes*. *Nepenthes* belong to the monotypic family of *Nepenthaceae*. Various digestive proteins have been identified in the pitcher fluid. For many of which their corresponding genes were cloned, heterologously expressed and further characterized. Besides hydrolytic activities, some of these proteins show antimicrobial properties. Although there is an increasing number of reports on the protein compositions of the fluid in *Nepenthes* species, our knowledge about the molecular regulation of the protein secretion is still limited. We are interested to learn more about the regulation of the protein composition on the molecular level. We found changes in endogenous phytohormone levels during prey digestion and induction of some pathogenesis-related protein (PR) gene expression after salicylic acid (SA), or jasmonic acid (JA) application. To get more inside into this carnivory process, we approach promoter analysis for selected genes. In order to get promoter region sequence information of such genes the Genome walking strategy is used. With this method we investigate regulatory elements of genes from pitcher fluid proteins which belong to the PR-family such as PR1. By analyzing identified regulatory elements we will not only study the regulation of single PR genes during prey digestion in *Nepenthes* but also try to identify cues for this carnivory signaling.

## The dark matters: A below ground herbivore drives the evolution of root secondary metabolites in nature

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Plants produce diverse and variable blends of toxic secondary metabolites in their shoots and roots. Intraspecific variation in secondary metabolite abundance may be explained by fitness benefits under herbivore attack that result in divergent selection patterns driven by the spatial heterogeneity of herbivores. To date, few studies have linked herbivore-dependent fitness benefits of a metabolite with patterns of natural selection and the resulting geographic differentiation, especially in below ground environments. Here, we show that a major root herbivore in central European grasslands, the white grub *Melolontha melolontha*, can shape both the phenotypic and genetic variation in root secondary chemistry of its native host plant, the common dandelion (*Taraxacum officinale*). By combining natural variation, genetic manipulation and chemical complementation, we show that the sesquiterpene lactone taraxinic acid  $\beta$ -D-glucopyranosyl ester (TA-G) deters *M. melolontha* and thereby directly decreases root damage and increases plant fitness. We further demonstrate that variation in *M. melolontha* abundance can lead to both phenotypic and genetic differentiation of TA-G concentration in the field through *M. melolontha* imposed induction and divergent selection. By combining 20 years of *M. melolontha* recordings with the characterization of natural *T. officinale* populations and their offspring, we show that high *M. melolontha* abundance is directly associated with high phenotypic and genotypic TA-G concentration in nature. Thereby, our experiments provide a causal link between the toxicity of a plant metabolite, its herbivore-dependent fitness benefits and its herbivore-driven variation in natural plant populations. Our study reveals the importance of below ground herbivores for the ecology and evolution of root secondary metabolites and highlights how insect herbivores shape phenotypic and genetic variation in plant secondary metabolites in nature. Future studies will investigate the mode of TA-G toxicity.

## Through the Looking-Glass, and What *Spodoptera* Found There – Detoxification of Maize Chemical Defenses

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Benzoxazinoids (BXDs) are indole-derived chemical defenses widespread in grasses (Poaceae). They are stored as stable glucosides and, upon damage, are hydrolyzed by specific  $\beta$ -glucosidases and converted into toxic aglucones. However, several Lepidopteran species adapted to be able to use grasses as a food source with different degrees of success. Our aim is to investigate the metabolic fate of BXDs in caterpillars and gain insight about the strategies they employ in order to cope with this family of maize chemical defenses.

By analyzing the BXD profile in frass of caterpillars feeding on maize leaves, we found the non-toxic DIMBOA-Glc (the major BXD in maize) and MBOA-Glc (a glucosylated derivative of the BXD degradation product MBOA). Among the species we studied, only *Spodoptera frugiperda*, *S. littoralis*, and *S. exigua* presented DIMBOA-Glc in the frass. Gut tissues showed UDP-glucosyltransferase (UGT) activity *in vitro* and the excreted compound was characterized as (2S)-DIMBOA-Glc, an epimer of the plant-derived (2R)-DIMBOA-Glc. Furthermore, we found that the insect-derived (2S)-DIMBOA-Glc is not hydrolyzed by plant glucosidases. Thus, the stereospecificity of the insect UGT towards DIMBOA, which exists as a racemic mixture, is crucial for its detoxification role. Transcriptome analyses allowed us to identify UGT genes from *S. frugiperda*, which are currently being expressed in cultured insect cells for activity screening towards DIMBOA and MBOA.

## Legume Chemistry and the Specificity of Pea Aphid Host Races

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The pea aphid (*Acyrtosiphon pisum*), a phloem sucking insect, has undergone a rapid radiation together with the domestication and anthropogenic range expansion of several of the legume host plants. Nowadays the pea aphid is a complex of at least 11 genetically different host races each specialized on a specific legume plant species such as alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), and pea (*Pisum sativum*), which we investigate in more detail. This ecological specialization can be considered as a first step towards sympatric speciation since the host fidelity of host races leads to assortative mating which reduces gene flow among them. The role of the host plant chemistry for this adaptation has not been studied so far, so the primary objective of this project is the identification and evaluation of plant chemical compounds involved in pea aphid-host plant specialization.

To identify the plant chemical compounds, we used a mass spectrometry-based non-targeted metabolomic approach. Although the plant species are quite closely related phylogenetically, the metabolic profiles of the uninfested plant species differ substantially, each plant species having a set of unique compounds. These might be important for the pea aphid–plant adaptation. By comparing the metabolic profiles of each plant species either uninfested or infested with different aphid host races, we observed not only a change of the metabolome upon aphid infestation but also a change depending whether an adapted or a non-adapted aphid race was feeding on the plant. We could find metabolites which are induced by non-adapted aphid races as well as features that are downregulated by adapted aphid races. These metabolites might also be important for the aphid–plant interaction. Based on these results we will isolate and purify the most likely metabolites and evaluate the response of the aphids against these metabolites in artificial diets.

## Harmonine – the defense alkaloid of the Asian lady beetle *Harmonia axyridis*

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More than 5200 species belong to the lady beetle family (Coccinellidae). Although a great variety of biological habitats and food preferences is visible, most of these beetles are carnivorous feeding on aphids, coccids and mites. Therefore species like *Harmonia axyridis* have been used as biological control agents in many countries but have become invasive threatening the native lady beetle assemblage. Common to all lady beetles is the so-called reflex bleeding: as soon as a beetle is disturbed, it exudes droplets of hemolymph from the tibio-femoral joints of its legs. The repellent and sometimes toxic properties of this fluid derive from contained alkaloids. The main defense alkaloid of *H. axyridis* is harmonine ((17*R*, 9*Z*)-1,17-diaminooctadec-9-ene). It shows a broad activity spectrum and displays amongst others antibacterial activity against fast-growing mycobacteria, *Mycobacterium tuberculosis* and *Plasmodium falciparum*. With ongoing research more and more properties of harmonine are discovered raising the interest in larger quantities of synthetic harmonine and derivatives to develop new lead compounds for medicinal use or industries. Therefore we designed a short and flexible synthesis of racemic harmonine *via* reductive olefination of a macrocyclic lactone derived from cyclooctanone. By enantioselective saponification of the lactone both enantiomers of harmonine become available in high yield. Through minor changes of the synthetic route, closely related derivatives can be obtained. Recently, racemic and enantiopure harmonine as well as a derivative showing one alcohol group instead of a secondary amino group have been synthesized. The synthesis of the non-natural (*S*)-enantiomer of harmonine is currently conducted. As soon as the (*S*)-harmonine is available bioassays will be performed clarifying whether both enantiomers of harmonine show the same activity spectrum or if there is a noticeable difference between the enantiomers.



## ***Spodoptera littoralis* detoxifies neurotoxin 3-nitropropanoic acid through conjugation with amino acids**

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*Spodoptera littoralis* is a phytophagous generalist. The host range covers more than 40 plant species that produce 3-nitropropanoic acid (3-NPA), an irreversible inhibitor of mitochondrial succinate dehydrogenase. Feeding on an artificial diet with admixture of 3-NPA significantly slowed larval growth but did not cause an increase in mortality. In contrast, injection of the same amount of 3-NPA resulted in acute toxicity and death. To study the detoxification metabolism of 3-NPA in *S. littoralis*, the frass of treated larvae were analyzed by HPLC. Amino acid amides of 3-NPA were identified as the main products of 3-NPA metabolism in the gut. Injection of isotopic labelled 3-NPA into the hemolymph provides evidence that 3-NPA is incorporated into amino acid amides. The detoxification products showed identical retention times compared to the synthetic amides of 3-NPA and glycine, alanine, serine and threonine. The toxicity of the amides was negligible as shown by injection of the synthetic standards. Bioassays provide evidence that the detoxification products are formed in epithelial cells of the gut tissue. Finally, the amides are transported to the hemolymph and excreted into the gut lumen. Proposed pathway of 3-NPA detoxification under further investigation.

## You are what you eat – Can selective advantages explain the AT-bias of endosymbiotic genomes?

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Endosymbionts are expected to evolve a reduced metabolic burden they impose on their host. Strikingly, both plasmids and intracellular bacterial symbionts generally exhibit a reduced GC-content relative to the chromosome of their host. The mechanistic causes for this pattern, however, remain poorly understood. We hypothesized that competition among cytoplasmic elements for the hosts' nucleotides may explain this observation: since dATP and dTTP are the most abundant nucleoside triphosphates in a cell, intracellular symbionts with lower GC-contents should be selectively favoured. Here, we test this hypothesis by experimentally manipulating the GC-content of plasmids and subsequently analysing the fitness consequences for the bacterial host. Specifically, we introduced eight 1 kb sequences of eukaryotic DNA that were particularly AT- and GC-rich into two minimal plasmid backbones. Growth experiments with *Escherichia coli* revealed a significant fitness decrease of cells that harboured GC-rich plasmids, thus supporting the above hypothesis. Moreover, quantitative real-time PCR revealed a significantly reduced copy number of GC-rich plasmids, which is consistent with a depleted pool of GC-nucleotides due to the presence of the plasmid. Furthermore, externally supplying GC-nucleotides to a coculture of both plasmid-harboring strains caused a growth increase of cells containing GC-rich plasmids, while feeding of AT- nucleotides did not cause a similar effect. This observation implies a shortage of GC-nucleotides in cells containing GC-rich plasmids. Our results demonstrate that by altering their base composition, plasmids can reduce the metabolic burden they impose on their bacterial host, which may explain the commonly observed GC-bias of plasmids. Future work will focus on the determination of intracellular nucleotide levels by flame ionization mass spectrometry. Furthermore, RNA Sequencing experiments are planned to investigate the effect of GC-nucleotide depletion on the cell's transcriptome with a special focus on nucleotide biosynthesis genes.

## **The bacterial network: Nutrient exchange via nanotubes**

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Bacterial interactions often involve cross-feeding individuals who supplement each other's nutritional requirements. In case of an inter-cellular exchange of metabolite there is a possibility of product loss via diffusion or unintended third party uptake. Cells could circumvent this loss by applying a contact dependent method of exchange. We have generated a cross-feeding system between *Escherichia coli* and *Acinetobacter baylyi* to demonstrate nutrient exchange via nanotubes. The two distant species show exchange of cytoplasm by connecting to each other through membrane derived structures referred to as nanotubes. Additionally the exchange is dependent on the nutritional status of the cell, thus indicating that the exchange may primarily serve to satisfy the metabolic requirements of the nanotube-forming cells. Altogether our results demonstrate that bacteria can use nanotubes to exchange nutrients among connected cells. This mechanism helps distribute metabolic functions within microbial communities and suggest the presence of a connected biochemical network used for growth.

## Timing is Everything: Transcription Speed and Stress Response in *Escherichia coli*

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For bacteria, and indeed for all living beings, fast adaptation to changes in the environment is a key to individual fitness and survival. In *Escherichia coli* a changing DNA supercoiling level is a primary prerequisite for responses to stress, like uptake shift or biofilm formation. How fast the RNA polymerase (RNAP) travels along a gene directly influences the supercoiling level (and vice versa), but similarly important, the RNAP speed determines a time lag between the regulation of a promoter's activity and an actual change in the expression level of the associated gene.

From RNAP ChIP-chip, transcriptome and RNA turn-over data, we have calculated the speed of RNAP over all expressed genes in *E. coli* and can show a significant enrichment of ontological groups in the fastest ten percent. Not surprisingly, these enriched groups encompass the core biosynthetic and gene expression machinery. With further group-wise comparative analysis we will recover the priority of different stress response steps embodied by the genes of these groups. A linear model will aid in relating the different RNAP speeds to genetic determinants, expanding on the already found correlations with genetic factors such as tRNA Adaptation Index (tAI) and relative codon frequencies.

## Geographical stability of an ecologically important gut microbiota pine weevils

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Nutritional symbiosis, in which microorganisms provide their insect host with nutrients are widespread in nature. However, nutritional mutualisms are not restricted to the synthesis and provision of essential aminoacids or vitamins but also encompass the degradation of noxious compounds in the host's diet. This has been demonstrated in fungal-insect associations. However, although long hypothesized, it remains poorly described for bacterial-insect associations.

The large pine weevil (*Hylobius abietis*) feeds on the bark and cambium of conifers where it encounters high amounts of complex resin acids, a form of conifer chemical defenses. These compounds are known to act as deterrents, and can cause neural damage and gut membrane disruption in insects. We are interested in studying what enables the pine weevil to exploit conifers as a food source and whether its gut microbiota is involved in the degradation of terpenoids.

Our observations suggest that terpenes not only do not exert a negative effect on the pine weevil, but that they seem to be beneficial. In order to explore the role of the microbiota on the degradation of these compounds we have characterized the gut microbial community via next-generation sequencing. We observe a geographically stable microbiota across different European populations dominated by Enterobacteriaceae (Proteobacteria) and Firmicutes. To place the bacterial community into a greater ecological context, we have compared it to that of other beetles exploiting similar and different ecological niches. We observe that the gut microbial community of the pine weevil is very similar to that of conifer-exploiting beetles particularly within the Enterobacteriaceae family.

To explore the functional aspect of this microbial community we have performed bioassays with beetles that have been depleted of bacteria using an antibiotics treatment. We observe that bacteria-free individuals do not digest terpenes as efficiently as untreated ones and that supplementation of the native community recovers digestion efficiency.

Collectively, our results suggest that the gut bacterial community of the pine weevil is essential for the exploitation of its ecological niche.

## Correcting mass errors: Computational recalibration of mass spectrometry imaging data

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Mass Spectrometry Imaging (MSI) has recently become widely popular because of its potential to map the spatial distribution of thousands of compounds in a single measurement directly from complex tissue surfaces, without the need of special labeling compounds. With its increasing use, large amount of data is generated routinely. Given the unique nature and large size of MSI data, efficient data processing strategies are critical to interpret results with higher confidence. With every MSI experiment, it is important to maintain high mass measurement accuracy for accurate identification of the observed ions. Many times this can be compromised due to different experimental factors. Herein, we introduce a novel procedure for lock mass-free recalibration, which is based on the assumption that spectra acquired from adjacent spatial locations represent similar molecular composition as well as the spectra may also exhibit similar mass deviation effects. This method performs mass shift correction of individual spectrum in the dataset, one at a time, by iteratively building a crystal of pixels using spectra acquired at individual coordinate positions.

We applied our method to the data obtained from laser-assisted desorption/ionization (LDI)-TOF MSI of surface lipids on intact *Drosophila melanogaster* flies. In this experiment, intact flies were fixed on a target plate. For this data, we observed mass deviations predominantly between  $\pm 0.3$  Da and  $\pm 0.5$  Da. Applying our recalibration method, observed mass deviations in individual mass spectra were strongly reduced. Lock mass correction of MSI data is difficult as not all spectra contain the selected peak. Our method eliminates this need.

## **Native root-associated bacteria protect their host plant from a fungal sudden-wilt disease via Induced Systematic Resistance and allelopathy**

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Plants maintain extensive microbial associations whose functions remain largely unknown. In 2011, our model plant *Nicotiana attenuata* suffered sudden tissue collapse and black roots, symptoms similar to a *Fusarium-Alternaria* disease complex, when grown in its native habitat, the Great Basin Desert, Utah, USA. To find potential remedy for this sudden wilt disease, 3 different protection strategies (fungicide, soil amendment and seed inoculations with native root-associated bacterial and fungal strain isolated from previous experiments) were tested in the field. A field trial with more than 900 plants in field plot showed that only the inoculation treatment with a mixture of five native bacterial isolates significantly reduced disease incidence and mortality. Similar disease reduction rates were obtained from a second field trial during the following year, demonstrating the robustness of the plant protection effect by bacterial treatment. In general, beneficial bacterial mutualist protects their host plants via different mode of actions such as Induced Systematic Resistance (ISR), production of antimicrobial and lytic enzymes, competition for nutrients and colonizing ecological niches. ISR mechanism is induced by root associated beneficial microbes by priming the whole plant body for enhanced defense against wide range of phytopathogens. Further investigations on the potential mechanism of disease suppression revealed that the mixture of bacterial isolates protect the plants by two synergistic mechanisms: by Induced Systematic Resistance (ISR) and by production of the antifungal compound surfactin by one of the bacterial isolates *Bacillus mojavensis*. Native plants, perhaps like most eukaryotes, develop opportunistic mutualisms with prokaryotes which help them to protect from phytopathogens.

# Poster



## **Confusion by the background: Try to smell your sex partner!**

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Finding a mating partner is an essential task for insects in order to ensure reproduction. Like in many other insects, females of the noctuid moth *Heliothis virescens* therefore release chemical cues, a species-specific pheromone blend, to attract conspecific males. In their environment male moths do not only detect the female sex pheromone blend, but they are also confronted continuously by a wide range of different odor molecules, mostly plant odors. We addressed the question, how a plant odor background influences pheromone-guided flight behavior in our wind tunnel, while using a common plant compound, linalool. Therefore, we analyzed the pheromone attraction of six different *Heliothis virescens* strains and selected one for the following experiments. Male moths showed strain-dependent attractivity strength towards the artificial pheromone blend in our wind tunnel experiments. Moreover, we investigated the influence of variation in pheromone plume structure on pheromone-guided flight behavior. Finally, we examined the attractivity of linalool for male moths, while using different concentrations. When males were stimulated simultaneously with the specific sex pheromone and the plant-related compound linalool, their pheromone-guided behavior was significantly suppressed in a concentration-dependent manner, while linalool alone elicited no behavioral response.

## Laser-assisted methods for spatially-resolved metabolomics

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Laser ablation electrospray ionisation (LAESI) is an ionisation technique for mass spectrometry (MS) working at ambient pressure and with minimal sample preparation. It is very well suited for MS imaging of biological samples with good spatial resolution, so the distribution of molecular targets in a complex sample such as a leaf or a microtome slice can be mapped. In this project, a simple LAESI ion source is to be constructed allowing for MS imaging in a cell-by-cell fashion within a complex sample, while sample preparation should be equal to preparing a slide for light microscopy. Possible applications are metabolomic analysis of plant and animal tissues, chemical mapping and exploration of intercellular interactions, as well as correlation imaging together with other imaging techniques. Focussing of the infrared laser to spot sizes below 100  $\mu\text{m}$  is to be achieved with infrared optics currently being developed by Norbert Danz at IOF. Assembly and integration of the other parts is scheduled to happen as fast as parts can be distributed by the manufacturers. Parameters that need to be determined experimentally are precise location of the focal plane and optimal amount and intensity of laser pulses used for sample ablation. Multiple device geometries need to be explored. With basic optimisation achieved, automation of the procedure as well as development of an analytic method can be tackled, to provide a convenient and powerful tool for elucidation of current questions in chemical ecology.

## A pollen´s journey: Which pollinator offers the best lift?

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The main pollinators of the wild tobacco plant *Nicotiana attenuata* are the hawkmoth species *Manduca sexta*, *Manduca quinquemaculata* and *Hyles lineata*, as well as the hummingbird species *Archilochus alexandri*. These floral visitors differ in their pollination behavior. Whereas day-active hummingbirds pollinate in the vicinity of their nesting sites, thus only transfer pollen within one population (short-distance transfer), it is believed that hawkmoths fly over long distances during night and as a result act as pollen vectors between populations (long-distance transfer). Moreover, these pollinators handle flowers differently and show different responses to a variety of floral traits such as floral scent or nectar. It is assumed that these various behaviors could entail differences in pollination and thus in plant´s fitness, especially concerning the outcrossing rate of the plant.

Based on these assumptions, the first aim of this project is to find out which pollinator transfer the pollen the best. Or with other words: Who offers the best lift? Therefor we will use two different locations of transformed plants to measure pollen flow within and between these locations by analyzing capsule and seed production of antherectomized *Nicotiana attenuata* mothers caused by the different pollinators. To distinguish between short- and long-distance transfer paternal plants of only one location are allowed to flower at the same time as the maternal plants of both locations, so that we are able to say from where the pollen was transferred. In order to find out who the pollen vector was, we will exclude either day or night pollinators by covering the plants. Additionally, we will measure seed production after single pollination events by different floral visitors.

The second aim of this project is to investigate which floral traits are associated with pollen transfer over short and long distances. For this purpose, we use different transformed paternal genotypes altered in the production of floral scent, floral nectar or both. Seeds produced by the maternal plants will be genotyped by using PCR and specific markers for the different genes knocked down in the used transformed lines.

## The roles of gene duplication in the evolution of anti-herbivore defenses in *Nicotiana attenuata*

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Gene duplication is one of the most important mechanisms for the evolution of novel traits. The genus *Nicotiana* includes 75 species distributed in wide range of habitats and have evolved various specialized anti-herbivore defenses, such as nicotine, multiple-domain trypsin protease inhibitor (TPI), and herbivore induced defense signaling. Although the ecological functions of these traits have been revealed using *N. attenuata*, an ecological model system, how did these traits evolve remain unknown. Gene duplication has been shown as one of most important mechanisms for the evolution of new gene functions and novel traits. Here, we hypothesize that gene duplication contributes to the evolution of *Nicotiana* specific traits. To test this, we performed both un-targeted and targeted analysis based on the characterized gene duplication history of 11 dicot plant genomes, including four recently sequenced *Nicotiana* species. For the un-targeted analysis, we identified all genes that were specifically duplicated in *Nicotiana*. Among them, several genes were involved in nicotine biosynthesis, indicating that the duplication events of these genes in *Nicotiana* might have contributed to the evolution of nicotine biosynthesis. For the targeted analysis, we characterized the gene duplication history and post-duplication expression changes of genes involved in nicotine biosynthesis, and anti-nutritional defense traits, e.g. TPI. Furthermore, we found that genes that were originated from the *Solanaceae* whole-genome duplication (WGD) were significantly enriched in the herbivore induced early defense signaling in leaves and roots. Taken together, our data showed that gene duplication contribute to the origin and evolution of anti-herbivore defense traits in *Nicotiana*. Currently we are working on the hypothesis that gene expression changes followed by WGD in *Solanaceae* contributes to the origin and evolution of both anti-nutritional defense traits as well as nicotine biosynthesis.

## **Chemical Defense responses of *Arabidopsis thaliana* to infection by *Sclerotinia Sclerotiorum***

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Phytoalexins as general antimicrobial compounds are synthesized by plants in defense against pathogens such as fungi. Fungi can however also produce enzymes that metabolize and detoxify these plant chemical defenses. A large number of studies support the idea that the ability of phytoalexins biotransformation is an important virulence factor for many fungal species such as *Botrytis cinera*, *Phoma medicaginis* and *Sclerotinia sclerotiorum*. The phytopathogenic fungus *S.sclerotiorum* is a devastating necrotrophic fungal pathogen that can cause stem rot disease in a vast range of plant species and results in large losses of crop yields worldwide. Currently, enzymatic detoxification of phytoalexins by *S.sclerotiorum* is of great interest due to its potential application to control this plant pathogen.

In this study, changes of defense compounds (glucosinolates, flavonols and camalexin) in *A.thaliana* after inoculate with two different *S.sclerotiorum* strains (Sequenced strain Uf-70 and isolate strain Ss-C) has been investigated. HPLC and LC-MS analyses showed that the concentration of these three compound classes rose in *A.thaliana* after infected by the two strains. But several days after inoculated with Uf-70, the concentration of flavonols and camalexin began to decline in *A.thaliana* which is in contrast with Ss-C. This result may suggest that *A.thaliana* can synthesize and accumulate these defense compounds in response to *S.sclerotiorum* infection whereas the pathogen has the potential to degrade these compounds for survival.

The candidate genes coding for enzymes involved in detoxification in *S.sclerotiorum* will be identified. Functional research of these genes will allow more insight to detoxification of defense compounds and virulence of *S.sclerotiorum*.

## Poplar responses to simultaneous herbivore and pathogen attack

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In their natural environment, plants face a multitude of simultaneously occurring antagonists. However, plant defense responses are mostly investigated with one attacking species only. In order to study the responses of a woody plant species to more than one enemy, the volatile emission and leaf chemistry of black poplar (*Populus nigra*) trees was analyzed after single and combined infestation by two different natural enemies of this tree species: the biotrophic leaf rust pathogen *Melampsora larici-populina* and larvae of the generalist-feeding lepidopteran *Lymantria dispar*. Caterpillar feeding significantly induced the emission of all major volatile groups. In contrast, pathogen infestation reduced the overall amount of emitted volatiles both alone and in combination with herbivory, and changed the composition of the odor blend. Future experiments, including RNA sequencing and herbivore choice assays, will give more insight into the molecular regulation of this interaction as well as its ecological consequences in natural black poplar populations.

## Unravelling 2-deoxy-2-fluoro-D-glucose metabolism in plant tissue using mass spectrometry and NMR

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### Introduction:

2-Deoxy-2-Fluoro-D-glucose (FDG) is a structural glucose analogue which is commonly used as a radioactive glucose surrogate in clinical diagnostics and animal studies to trace uptake and metabolism of glucose. FDG mimics the glucose distribution and it was assumed that after uptake, it is metabolized via glycolysis pathway to FDG-6-phosphate but not further. However, numerous papers describe the fate of FDG to FDG-6-P and further metabolites in the animal cells. FDG has also been employed in plant radiotracer studies but its metabolism in plant cells is not yet characterized. Elucidating FDG metabolism in plants is a crucial aspect for establishing its application as a radiotracer in plant imaging. Here, we describe the metabolic fate of FDG in model plant species, *Arabidopsis thaliana*.

### Aims:

To elucidate FDG metabolism in plants.

### Experimental method:

Mature leaves of *A. thaliana* (short day plants, 6-7 week, early flowering stage) were gently pricked on the abaxial surface. Five microliter of FDG (20 mg.mL<sup>-1</sup>) was immediately applied in the pricked spots. Four hours later leaves were extracted using Chloroform:Methanol:Water (1:2:1). Aqueous fraction was analyzed by LC-MS/MS and NMR for the presence of <sup>19</sup>F-containing compounds.

### Preliminary results:

LCMS and direct infusion MS results confirmed the presence of 5 different <sup>19</sup>F containing metabolites in the extract. In total, we putatively identified above <sup>19</sup>F containing metabolites as FDG (*m/z* 181.0513), F-gluconic acid (*m/z* 197.0464), FDG-6-P (*m/z* 261.0180), F-maltose (*m/z* 343.1051), and UDP-FDG (*m/z* 567.0434) on the basis of known literature information, their exact mono-isotopic mass ( $\pm$  5 ppm mass error) and MS/MS fragmentation analysis. Characterization of purified compounds using NMR confirmed identification of <sup>19</sup>FDG-6-P (*m/z* 261.0180), and <sup>19</sup>F-maltose (*m/z* 343.1051) as major end products of <sup>19</sup>FDG metabolism in *A. thaliana* leaf cells.

### Current focus:

Phloem exudate analysis for identifying FDG transportation form in plant vasculature.

### Future prospects:

FDG cytotoxicity studies.

Detection of low abundance <sup>19</sup>F-metabolites.

## **The absolute configuration of salicortin, HCH-salicortin and tremulacin from *Populus trichocarpa x deltoids* Beaupré**

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The absolute configuration of salicortin, HCH-salicortin and tremulacin isolated from leaves of *Populus trichocarpa x deltoides* Beaupré, was determined by comparison with spectroscopic data from idescarpin, isolated from leaves of *Idesia polycarpa*. All compounds were characterized by nuclear magnetic resonance spectroscopy, high-resolution mass spectrometry and circular dichroism spectroscopy. It was found that the hydroxyl cyclohexenonyl (HCH) moiety in all compounds is (*S*)-configured. In addition it was shown that leaves of *Idesia polycarpa* contain idescarpin in high amount (1.09%, based on dry weight).



## Crystallization and Structure analysis of a *Phaedon cochleariae* Isopropyl diphosphate synthase (*PclDS1*)

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### Introduction

Isopropyl diphosphate synthases (IDS) are ubiquitous enzymes that catalyze consecutive condensation reactions in the isoprene biosynthetic pathway, yielding short-chain prenyldiphosphates (C<sub>10</sub>, C<sub>15</sub> and C<sub>20</sub>), which are the precursors of monoterpenes, sesquiterpenes and diterpenes, respectively. Generally, different IDSs produce single products. However, recently a bifunctional scIDS (named *PclDS1*) has been reported from the mustard leaf beetle, *Phaedon cochleariae*, which can control different product-length by different metal cofactors. Specifically, *PclDS1* produces about 96% geranyl diphosphate (GDP, C<sub>10</sub>) and only 4% farnesyl diphosphate (FDP, C<sub>15</sub>) in the presence of Co<sup>2+</sup> or Mn<sup>2+</sup>, whereas it produces 18% GDP and 82% FDP in the presence of Mg<sup>2+</sup>. While the precursor GDP has been shown to be essential for the synthesis of defensive terpenoids, FDP may serve as precursor for various primary metabolites and juvenile required hormone in the beetles. The direction of flux at a branch point in terpene metabolism between defense and primary metabolism is regulated by an unprecedented IDS control mechanism. However, the molecular mechanism of this bifunctional catalytic activity of *PclDS1* is completely unknown to date.

Consequently, the aim of my PhD project is to illuminate the mechanism of divalent metal-mediated product chain-length determination by mutagenesis and protein structure determination by X-ray crystallography.

### Work plan

Construction of pET100-SUMO-*PclDS1* recombinant plasmid and its heterologous expression in *E. coli* BL21(DE3)star strain; Optimizing the culture conditions, protein capture and intermediate protein purification, and SUMO(+)-*PclDS1* and SUMO(-)-*PclDS1* enzyme activity testing; SUMO(-)-*PclDS1* polishing (purity > 95%, concentration > 1mg/ml) and crystal formation in the presence of different substrates, and different divalent metal ions; X-ray diffraction of crystals from wild type protein and *PclDS1* mutants.

Note: A has already finished, B is under investigation, C. and D. will be done in future.

## Different biosynthetic routes leading to the formation of 2-phenylethanol in *Populus trichocarpa*

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Plants are under a continuous influence of biotic and abiotic stresses. As a defense against herbivores, many plants emit a complex blend of volatile compounds upon attack of herbivores. These volatile compounds comprise terpenes, green leaf volatiles, nitrogenous compounds and aromatic compounds. Plant volatiles can reduce the fitness of the herbivore on the emitting plant as they might attract herbivore enemies. Recently we identified several enzymes responsible for the formation of nitrogenous volatiles in poplar. We showed that L-phenylalanine can be converted by CYP79 enzymes to benzyl cyanide with phenylacetaldoxime as an intermediate. RNAi-mediated knock-down of the responsible *CYP79* genes in *Populus canescens* resulted in reduced amounts of phenylacetaldoxime and benzyl cyanide. Surprisingly, the abundance of 2-phenylethanol, a volatile alcohol, was dramatically decreased as well. To study the formation of 2-phenylethanol in more detail, we conducted a series of labeling experiments using deuterium-labeled potential pathway intermediates. Feeding of labeled (*E/Z*)-D<sub>5</sub>-phenylacetaldoxime to detached poplar leaves resulted in the emission of labeled 2-phenylethanol, benzyl cyanide, and 2-phenylacetaldehyde, whereas upon 2-D<sub>4</sub>-phenylethylamine feeding only labeled 2-phenylethanol and 2-phenylacetaldehyde were present in the volatile blend. These results suggest that there are two separate pathways for the biosynthesis of 2-phenylethanol formation in poplar. In aim of unraveling the enzymatic reactions involved in these putative pathways, we already identified a nitrilase (PtNIT1) that converts benzyl cyanide to phenylacetic acid, which might be further reduced to 2-phenylacetaldehyde. In addition, we characterized four aldehyde reductases (PtPAR1, 2, 4, 5) that were able to accept 2-phenylacetaldehyde as substrate. Interestingly, the aldehyde reductases were also able to convert fatty acid derived aldehydes like nonanal and the monoterpenes citral and citronellal into the corresponding alcohols. The characterization of the two biosynthetic pathways for volatile alcohol formation will lead us to a better understanding of plant defense and possible applications in pest control.

## Benzoxazinoids: Biosynthesis and function of major defense compounds in maize

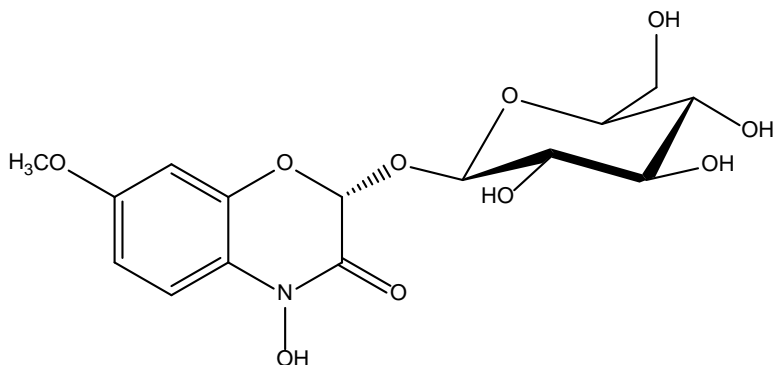
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Benzoxazinoids (BXDs) are major defense compounds in grasses and act against fungi, aphids, and caterpillars. The biosynthesis of benzoxazinoids is well established leading to DIMBOA-Glc (2-(2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one)- $\beta$ -D-glucopyranose, compound 1). However, several grass species like maize or wheat contain other BXDs such as HDMBOA-Glc (2) and DIM2BOA-Glc (3) whose biosynthesis and impact on plant defenses are unknown. We aim to identify the enzymes involved in the biosynthesis of 2 and 3 in maize using modern genetic approaches and to investigate the ecological functions of these compounds.

A quantitative trait locus (QTL) mapping of the content of 2 revealed a locus on maize chromosome 1. This locus comprises three O-methyltransferase genes (OMT) which were designated as Bx10a, Bx10b and Bx10c. Overexpression in *Escherichia coli* and subsequent assays with purified recombinant proteins showed that Bx10a-c were able to convert 1 to 2. A natural transposon insertion in Bx10c in some lines was found to correlate with decreased formation of 2 and an increase of callose deposition causing higher leaf aphid (*Rhopalosiphum maidis*) resistance. The QTL mapping of 3 revealed two loci containing a 2-oxoglutarat-dependent dioxygenase (2ODD) and an O-methyltransferase, respectively. Recombinant 2ODD catalyzed the oxidation of 1 in vitro, resulting in a precursor of 3. The characterization of the putative O-methyltransferase is currently underway



Chemical structure of DIMBOA-Glc - the predominant benzoxazinoid in maize seedlings.

## Smelling a low-cost meal: Hawkmoth use olfactory information to optimize their foraging behavior

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Hawkmoths and the flowers they pollinate are a classical model of coevolution since Darwin's times. There is a striking correlation between the length of a moth's proboscis and the length of the corolla tube of the flower the moth is mainly feeding from. However, it still remains unclear, whether the hawkmoth olfactory preference is similarly tuned to the volatiles emitted by the mainly visited flowers. Here we study the relationship between *Manduca sexta* and different *Nicotiana* species to explore the odor-guided behavior, which a moth might use to detect and pinpoint a suitable nectar source during flight. We ask, whether moths exhibit olfactory preferences for specific *Nicotiana* species, and whether the most preferred flowers are the most profitable ones for the moths. Plant species were selected to represent a large variety of flower sizes while still being potential nectar sources for *Manduca*. Gas chromatography and electrophysiological recordings were used to identify those flower volatiles that become detected by the moth. We then examined the behavioral response of *Manduca* towards these floral odors at the precise moment of odor plume encounter. To do so we tested the flight behavior of the hawkmoth in a wind tunnel using 3d- video tracking and an odor plume model based on real time volatile measurements. Finally, the net energy gain of *Manduca* foraging on the different flowers was calculated by balancing the energy gained through the nectar against the energy spent during flower handling. The amount of energy spent was obtained via online Co<sub>2</sub>-measurements during free flight. Our results suggest that *Manduca* prefers the volatiles of those flowers which also fit the length of its proboscis. These volatiles elicited a response profile on the antenna which further enhanced the chemical differences between these species and the less fitting ones. Moreover the odors of the best fitting plant species initiated a surge-link flight behavior by increasing flight speed and upwind heading. In contrast to this, the odor of the less fitting plants triggered a more casting-like behavior with reduced speed and increased crosswind flights. These differences in flight patterns resulted in more odor plume contacts with odors from fitting flowers and consequently in more odor source approaches. Finally, we show that the most preferred and best fitting flower also had the highest net energy gain. Interestingly the net energy balance did not only depend on the nectar energy provided by the flower, but was also strongly influenced by the costs of flower handling. Hence the coevolution of hawkmoths and flowers did not only determine the length of the moths' proboscis, but also resulted in an olfactory preference for those flowers promising the highest net energy gain.

## **CML9 – A calcium sensor protein involved in plant defense?**

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During their lifetime, plants have to deal with diverse biotic and abiotic stimuli. Regarding that, more than half of all insects on earth are herbivorous, thus feeding of insects is a major biotic stress factor for plants. As sessile organisms they have to react to these attacks to overcome them. Throughout evolution plants have developed various direct and indirect defense strategies. Until now, little is known about the recognition and the early signal transduction pathway leading to the appropriate defense response. Calcium ( $\text{Ca}^{2+}$ ) as a second messenger plays an important role in mediating such responses as it is one of the earliest components activated by insect feeding.  $\text{Ca}^{2+}$  elevations are ubiquitous signals originating from different biotic and abiotic stresses. The specific decoding of these signals is of great interest. In *Arabidopsis*, 250 calcium binding proteins are known, including sensor responders and sensor relays. Microarray data revealed that a group of sensor relays, the Calmodulin-like proteins (CMLs), are regulated upon herbivory. Recently it was shown, that several CMLs are inducible by the oral secretion of the generalist herbivorous insect *Spodoptera littoralis*. One of these regulated CMLs is *CML9* (*CAM9*). *CML9* is a calcium sensing protein with 4 EF-Hands (calcium binding domains). It has been reported to be induced by mechanical stimuli like touch and wounding and by biotic stress such as the attack of pathogens. This work aims to investigate the role of *CML9* in the interaction between *Arabidopsis thaliana* and herbivorous insects. Using MecWorm, a mechanical larva, it could be shown that both the mechanical damage caused by the insect and the elicitors in the insect oral secretion lead to the induction of *CML9*. To elucidate if *CML9* is involved in the plant defense pathway against herbivory further studies on the gene expression profile are needed as well as the analysis of *CML9* knock-out-lines.

## **Genetic background, ploidy and ovule-directed transcriptome analysis: a biogeographic approach to understanding apomixis penetrance in *Poa pratensis***

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Apomixis is asexual reproduction through seeds, leading to offspring that are genetically identical to the mother plant. Apomictic lineages are derived from sexuals and should therefore reflect the genetic background of their sexual ancestors, in addition to expected mutation accumulation (i.e. Muller's ratchet) over time. Multiple origins of apomictic lineages from a sexual gene pool will thus be reflected by both large (inter-lineage) and small (intra-lineage) genetic differentiation.

*Poa pratensis*, an important forage and turf grass characterized by variable ploidy, a huge genome and versatile reproduction mode, ranging from obligate sexual to facultative apomictic to obligate apomictic, is a useful model for testing these evolutionary processes. The spread of apomixis in natural populations leads to mixed populations with varying levels of apomixis penetrance. Furthermore, ploidy variation may represent an adaptive mechanism to complement deleterious mutation accumulation. The aim of the thesis is to test whether (i) genetic variation in 133 *P. pratensis* accessions from 29 different countries reflects multiple apomixis origins, (ii) polyploidisation is correlated with apomictic penetrance, and (iii) elevated genetic variation can be used as a tool for identifying conserved apomixis factors in ovule-specific transcriptome analyses.

A structure analysis of microsatellite data identified three different groups within the data set, but none of the tested biological parameters reflected the observed clusters. The distribution of pairwise genetic distances reflected that for a sexual, rather than asexual population. This leads to the hypothesis that even limited amounts of sexual reproduction, in combination with ongoing hybridization, can obscure biogeographic patterns of asexuality in *P. pratensis*, which is a globally distributed, facultative apomict. The reproductive mode and the extent of apomictically produced seeds (i.e. penetrance) in different genotypes will be determined using a Flow Cytometric Seed Screen. Additionally, 32 different *Poa* species will be examined using microsatellites and sequencing of two chloroplast genes in order to estimate kin relations and levels of hybridization. A completed ovule specific gene expression study using custom *Poa*-specific microarrays is being analyzed to test whether differences in ploidy and apomixis penetrance could be associated with differential gene expression.

## Navigation during long foraging paths of desert ants

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Desert ants *Cataglyphis fortis*, navigate individually in the salt pans of Tunisia by means of so called path integration (vector navigation). By combining, from a step integrator gained distance information with directional information mainly from celestial sources, the ant calculates a home vector to the starting point of the outbound run continuously (in central place foragers primarily the nest). However, as path integration is error-prone, the ants in addition use visual and olfactory cues to pinpoint their nest entrance. It has been shown that the accuracy of the path integrator as well as the ants' confidence in that egocentric navigational tool decreases with increasing foraging distance. Here we show that despite the accumulating errors, even after far-reaching foraging runs (>1200m way length) path integration provides the ants with surprisingly accurate information regarding the nest position. However, in addition the ants take also distant visual cues into account that are most probably provided by the patterns along the horizon line behind the nest entrance. So by artificially setting path integration and geocentric information in conflict, the experimentally displaced homing ants then took a compromise course between the geocentric and the egocentric information.

## Partners in Olfaction: the influence of SNMPs and OBPs on olfactory signaling in *Drosophila*

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The environment of insects is permeated with volatile molecules, from the odor blend of food sources and oviposition sites to repellent odorants or species-specific semiochemicals. Detection of these chemical cues via the sense of smell, i.e. olfaction, is crucial for survival and reproduction. Diverse stimuli have to be identified and evaluated in a context-dependent manner, even when available only in trace amounts. The fruitfly *Drosophila melanogaster* is a model organism amenable to behavioral, electrophysiological, genetic as well as neuroanatomical experimentation and analysis, and a popular system for the study of insect olfaction. Although a substantial amount of data on this species is available in the context of biochemistry, ecology, ethology, neurobiology and morphology, comparatively little is known about perireceptor events in the olfactory system. Beside the receptor there are multiple, additional proteins likely involved here. Odorant binding proteins (OBPs) are proposed to bind volatile, hydrophobic odorants enabling them to transition into the aqueous sensillum lymph surrounding the outer dendrite of olfactory sensory neurons (OSNs). Sensory neuron membrane proteins (SNMPs) are homologs of the vertebrate CD36 protein family: they are considered to be involved in activation and inactivation kinetics of olfactory signaling. However, the exact nature of the transport process and possible influence of the OBPs and SNMPs on specificity and sensitivity of OSNs is not clear.

I want to learn more about the contribution of SNMPs and OBPs to olfactory signaling and behavior. Using the example of OR88a I will analyze their influence on an olfactory receptor's ligand specificity. The receptor OR88a has a very distinct ligand profile in its natural sensillum environment, the trichoid sensilla type at3c. However, the deorphanization of OR88a by misexpression in the basiconic "empty neuron system" ab3a changes its ligand specificity drastically. Using laser dissection microscopy I will remove OSNs as well as supporting cells from trichoid sensilla housing OR88a expressing neurons and of basiconic ab3 sensilla. From the acquired small cell clusters I will extract RNA and perform transcriptome analysis as well as qPCRs. Characterizing the transcriptomics of both sensilla environments will help to validate the role of signaling factors, like SNMPs and OBPs, in olfactory signaling and identify potentially contributing proteins. Analysis of the corresponding mutant *Drosophila* lines in single sensillum recordings will allow me to assess the respective contribution of each protein to the ligand specificity of the at3c neuron. Finally the use of electrophysiological and biochemical methods will allow me to scrutinize the influence of isolated, single signaling factors on the activity of OR88a in a heterologous expression system.



## Small Molecule Signals in the soil dwelling nematode *Oscheius tipulae*

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The hermaphroditic nematode *Oscheius tipulae* is widespread and abundant in soil throughout the world with high levels of genetic diversity and large-scale geographical structure. *Oscheius tipulae* is commonly encountered as the dauer stage larva (a nonfeeding and highly stress resistant alternative larval stage optimized for long term survival) and therefore are not considered to proliferate in the soil. Furthermore, *O. tipulae* have been isolated from insect larvae of *Tipula paludosa*, indicating a necromenic life-style. Development and behavior of nematodes has been shown to be regulated by a class of nematode-derived small molecule signals - the ascarosides - glycolipids of the dideoxysugar ascarylose linked to fatty acid derived side chains. We aim to identify these components using a combination of NMR- and MS-based comparative metabolomics techniques along with bioassay guided fractionation. MS/MS-screening of the *O. tipulae* metabolome extract revealed the presence of ascaroside#9 as the dominating component, along with traces of two closely related homologs. We characterized ascaroside#9 in bioassays aiming at behavior (spot attraction assay) and development (dauer induction assay) using synthetic material. These experiments revealed that ascaroside#9 did not display dauer inducing activity in *O. tipulae*. However, our results suggested that biologically relevant concentrations of ascaroside#9 affect the nematode's behavior. Hermaphrodites of *O. tipulae* are repelled to nanomolar concentrations of ascaroside#9, whereas smaller concentrations have no effect.

## Extraction of stylar fluid after controlled mixed pollinations in *Nicotiana attenuata* and subsequent *in vitro* pollen tube growth bioassay

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### Abstract

The self-compatible plant *Nicotiana attenuata* grows in genetically diverse populations after fires. Despite naturally-available diverse pollen sources being equally proficient in siring seeds in single-genotype pollinations, self-pollen was consistently selected in mixed pollinations, irrespective of maternal genotype. However, clear patterns of mate discrimination occurred amongst non-self-pollen when mixed pollinations were performed soon after corollas open. In flowering plants, fertilization requires the delivery of the two sperms to each ovule via a male gametophyte structure, pollen tube. In plants with solid styles, pollen tubes grow in the intercellular space of the transmitting tract, and the growth requires the interaction between the gametophyte and the sporophyte. To investigate the mechanism of pre-zygotic mate selection in *N. attenuata*, it is thus crucial to develop a method of extracting the stylar fluid, which would include the molecular signals responsible for the pollen tube selection, and a subsequent reliable bioassay procedure to visualize the effect of stylar extracts on the *in vitro* growth of pollen tubes. We collected upper parts of the styles after mixed pollinations involving various pollen-pistil combinations. Subsequently, we vacuum infiltrated them and then centrifuged them in filter devices with 100 kDa cut-off at 14,000g to get enough yield for bioassay. Subsequent *in vitro* bioassay on modified pollen tube growth media presented a similar profile of pollen tubes performance to the *in vivo* performance predicted by the seed sets. We again vacuum infiltrated the freshly extracted styles or the same stored in -80°C freezer and recovered the extract by centrifugation to test if the molecular signal is obtainable after the first extract. The bioassay of these second-wash extracts did not show the predicted pollen tube performance as the first-wash extract did. However, the bioassay system needs to be improved for better reproducibility; the stylar fluid extraction method and the bioassay system are significant tool for further study of the interaction between gametophyte and sporophyte in flowering plant.

## Think Before Making a Decision

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Natural olfactory stimuli are often complex mixtures of volatiles, which might be mixtures of attractive and aversive odors, in which the identities and ratios of components are important for a fly to compromise and make a decision. Despite this importance, the mechanism by which the olfactory system processes and integrates this complex information remains unclear.

Combining behavioral experiments using the FlyWalk with neurophysiological experiments using optical imaging (from different orders of neurons), we sought to study how and where the information about odor valence, attractive and aversive odors, is encoded and ultimately integrated along the olfactory pathway.

*Drosophila* behaves differently when it is faced to different ratios of odor mixtures of attractive and aversive odors. The fly is strongly attracted to a mixture of attractive (Ethyl acetate, a food odor) and a well-known aversive odor (Benzaldehyde) with high concentration of attractive odor (Ethyl acetate, a food odor) compared to a mixture with low concentration of ethyl acetate.

On the brain level, the output level of the antennal lobe, the first olfactory processing center, is already involved in integrating attractive and aversive odors when the fly is faced to both simultaneously.

## To go or not to go?

### Behavioral responses to binary mixtures of attractive and aversive odors

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All animals are challenged by a huge amount of odors. Those can be of good (e.g. food related or sex pheromones), as well as of bad valence (e.g. predators or unhealthy food) for different organisms. In nature, these odorants of opposed valences can co-occur in mixtures and the animals have to decide for a compromise whether to tolerate the bad smell and go for the source or to refuse the odor source. In addition to the absolute valence of the odorants in a given mixture, the relative concentration ratios of positive and negative compounds may also influence the behavioral decision. However, up to date it remains unclear how and where along the olfactory pathway this decision is made. Therefore we investigate the olfactory guided behavior of *Drosophila melanogaster* in the FlyWalk to get insight in flies' decision for or against different attractant:repellent ratios, presenting the attractant ethyl acetate (component of balsamic vinegar) in binary mixtures with the repellent Benzaldehyde (found in almond). In addition we use calcium imaging to follow olfactory information along the pathway to highlight the spot of decision in the fly brain.

## Glucosinolate detoxification and sequestration – two beetles, two pathways

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Plants of the *Brassicaceae* family contain a myrosinase-glucosinolate defense system to chemically protect themselves against herbivory. Upon tissue damage, myrosinase (thioglucosidase) enzymes hydrolyze glucosinolates (gls), and the resulting products are released from the damaged plant tissues making them toxic for insects. However, some insect species have mechanisms to avoid such toxicity. We are investigating the stem flea beetle *Psylliodes chrysocephala* and the striped flea beetle *Phyllotreta striolata*, both specialist herbivores of crucifers, to determine how they handle glucosinolate defenses. We performed feeding experiments with <sup>14</sup>C-labeled 4msob (4-methylsulfinylbutyl)-gls and sinalbin (*p*-hydroxybenzyl-gls) to investigate how both beetles metabolize these glucosinolates. It was previously shown that *Phyllotreta striolata* sequesters glucosinolates with possible substrate preference to 4msob-gls. However, hydrolysis products were detected as in the feces and in the body after feeding on both glucosinolates, meaning that striped flea beetles may need further detoxification mechanisms as well. We found no radioactivity in the bodies of *Psylliodes chrysocephala*, in accordance with it having no sequestration mechanisms. It seems that stem flea beetle has several pathways for detoxification of glucosinolates. In the feces after <sup>14</sup>C-sinalbin feeding, desulfo-sinalbin was detected as a major metabolite together with several unknown conjugates. *P. chrysocephala* desulfates this glucosinolate, thus avoiding that plant myrosinases recognize it as substrate. On the other hand, *P. chrysocephala* fed on 4msob-gls excreted only two metabolites, the intact glucosinolate and the product of its hydrolysis – nitrile. Our ongoing investigation will identify the major unknown metabolites in the processes of sequestration and detoxification, which will shed light on novel aspects of host adaptation in this group of insects.

## Carbon Isotope Ratios Determination:

### Applying in vitro enzyme-catalytic model to in vivo study

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During the process of plant photosynthesis with three main pathways: C<sub>3</sub>, C<sub>4</sub> and CAM, carbon isotope discrimination occurs because of diffusion and carboxylation, led to less <sup>13</sup>C/<sup>12</sup>C ratios in plant tissue than that in the gaseous CO<sub>2</sub>. The further carbon metabolism additionally affects isotope ratios during metabolic and branching reactions and varies the isotopic signatures of metabolites from each other, such as terpenes, which are the largest and most diverse class of secondary metabolites. Chamomile is rich in terpenes and well known for its therapeutic purposes. A number of researchers studied the qualitative and quantitative terpene composition in chamomile, which is varied in different organs and different development stages, and the main terpene synthases were characterized recently. In this study, the terpenes in the volatile fractions and essential oils were obtained from the flowers, leaves, stems and roots at different development stages of *Chamomilla recutita* L. Rausch by *in situ* volatile collection and also organic solvent extraction followed by gas chromatography-mass spectrometry (GC-MS) analysis, while their corresponding carbon isotope ratio were also determined by gas chromatography-isotope ratio mass spectrometry (GC-IRMS). Combining with our previous study to in vitro enzyme-catalytic model, developed for tracing the biosynthetic pathway of terpenes, the isotope figure patterns of terpenes obtained from chamomile could be explained and clarified, which would also be extended to more plants and metabolic studies.

## Elucidating the molecular mechanisms in a defensive Beewolf-*Streptomyces* symbiosis

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Symbiosis is ubiquitous in our nature. A lot of focal points have been given to categorizing the kind of partnership and characterizing contributions of both the microorganisms and host to each other. So far, we learn that the bacterial mutualists can turn inaccessible biomass into nutrients or synthesize amino acids to feed the host, detoxify harmful food substances for the host, change host behaviour in favour of both partners etc. However, not much attention has been given to how partners were paired up to deliver mutualistic effect among many other microbial competitors. The European beewolf (Hymenopteran, Crabronidae, *Philanthus triangulum*) is a species of solitary, digger wasps which engage in a defensive partnership with Actinobacterium '*Candidatus Streptomyces philanthi*' (CaSP) inside specialized antennal gland reservoirs. Previous analyses showed that the host and symbionts have become partners from an ancient and single uptake, then co-diversified ever since with horizontal exchanges of symbionts across host species. Experimental infection of non-native bacteria into antennae of aposymbiotic female beewolves surprisingly resulted in a lack of vertical transmission. Taken together, these studies suggested some host mechanisms are maintaining a high specificity with the native symbiont. In my PhD project, these molecular mechanisms underlying partner choice will be studied. Our comparative transcriptome data has singled out some differentially expressed genes between aposymbiotic and symbiotic beewolf individuals, and preliminary testing of them pointed to some immune effectors as candidates for the partner choice of symbionts. A number of molecular methods, including RNAi, will be used to pinpoint the host determinant(s). An *in vitro* assay which aims to assess the sensitivity of CaSP and free-living *Streptomyces* to candidate immune effectors will be developed. Other than looking at the partner choice mechanisms, a state-of-the-art transposon-insertion deep sequencing would also be employed to discover the symbiont factors essential for colonizing the antennal gland reservoirs and elements possible for host recognition and vertical transmission.

## Social networking in the underground - an ectomycorrhizal story

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The rhizosphere of the ectomycorrhizal partners spruce (*Picea abies*) and *Tricholoma vaccinum* is a widely diverse habitat. Other soil organisms produce substances which can modulate the signaling and establishing of mycorrhiza. Therefore, we analyzed the signaling potential of *T. vaccinum* and other microorganisms at a sampling site using genome sequencing, proteomics and chromatographic approaches with the fungus as well as characterization of the soil community. The *T. vaccinum* genome was analyzed and 206 proteins were annotated. Protein sequences included small proteins with effector potential as well as enzymes for synthesis of the phytohormones salicylic acid, jasmonic acid, abscisic acid and indole-3-acetic acid (IAA). We isolated over 100 different bacteria and fungi during two years of sampling and checked them for growth promoting abilities towards *T. vaccinum*. As a potential morphogenic substance, the zygomycete soil fungal trisporoid intermediate D'orenone was investigated. . It reduced fungal growth and increased fungal IAA excretion *via* up-regulated transcription of a MATE transporter. Furthermore, D'orenone was found to modulate root architecture, increasing lateral root length. Thus, D'orenone provides interspecies and interkingdom signaling function in the ectomycorrhizal habitat. In conclusion, metabolites from soil microorganisms, like D'orenone, affect mycorrhizal signaling by changing phytohormone pattern. Analyzing the community culture-independent and with special focus on phytohormone production is in progress to improve the knowledge about signaling in the mycorrhizosphere.



