



24th IMPRS Symposium

April 2-3, 2025 Altes Schloss Dornburg, Max-Krehan-Straße 4, 07774 Dornburg

Please join us for:

- 2 guest talks
- 15 PhD talks
- 21 poster presentations
- 4 awards

Guest speakers



Sandra Irmisch

University of Leiden, The Netherlands Institute of Biology Leiden (IBL)



Ricardo Machado

University of Neuchâtel, Switzerland Institute of Biology Experimental Biology Research Group

Program

April, 2nd, 2025

	Travel by regional train from Jena Paradies to Dornburg/Saale RB 25 to Halle/Saale leaves at 8:19 in Jena Paradies (Jena Saalbahnhof 8:21, Jena- Zwätzen 8:24) and arrives in Dornburg at 8:31
09:15	Welcome (Großer Kaisersaal) <i>Claudia Voelckel</i>
09:30	New arrivals and defended theses in 2024/2025 (Großer Kaisersaal) Sarah O'Connor
09:45	Plenary Lecture 1 (Großer Kaisersaal) "Glowing belowground: What are the Ecological Functions of Bacterial Bioluminescence in Soil Ecosystems?" <i>Ricardo Machado, University of Neuchâtel, Switzerland</i> <u>Abstract</u> page 8 <i>Chair: Rayko Halitschke</i>
10:30	Coffee break (Rittersaal)

1 st talk session (Großer Kaisersaal)	
	, Chair: Linh Mai Nghat
11:00	1 - Cracking the genetic basis of green-brown polymorphism in meadow grasshopper (<i>Pseudochorthippus parallelus</i>) <i>Xinyi Chang (Population Ecology Group, FSU)</i> <u>Abstract</u> page 10
11:15	2 - Sources of variation in plant chemical diversity Linh Nguyen (German Centre for Integrative Biodiversity Research - iDiv)) Abstract page 11
11:30	3 - The chemical and sensory bases of social immunity in the clonal raider ant <i>Sandra Tretter (Lise Meitner Group Social Behaviour, MPI-CE</i> <u>Abstract</u> page 12
11:45	4 - The Not-So-Sweet Life of a Sweet Potato: Metabolic Changes Upon Mutualistic and Pathogenic Interactions <i>Yelyzaveta Zhyr (Research Group Plant Defense Physiology, MPI-CE)</i> <u>Abstract</u> page 13
12:00	5 - NOT Everything Everywhere All at Once: How Interdependent Symbionts in a Beetle Work Dongik Chang (Department of Insect Symbiosis, MPI-CE) <u>Abstract</u> page 14
12:15	Lunch break (Rittersaal)

1 st poster session (odd numbers)		
	Chair: Claudia Voelckel	
13:30	One minute poster talks (1,3,5,7,9,11,13,15,17,19) (Großer Kaisersaal)	
13:45 – 15:15	Poster session (Kleiner Kaisersaal & Flur)	
15:15	Coffee break (Rittersaal)	
15:15 – 15:45	IMPRS faculty meeting (Bibliothek 1)	

2 nd talk session (Großer Kaisersaal)	
	Chair: Jessil Ann Pajar
15:50	6 - From genes to ecosystems: ecological role and agricultural potential of gramine <i>Sara Leite Dias (Department of Molecular Genetics, IPK)</i> <u>Abstract</u> page 15
16.05	7 - Heads or tails? Sensory equipment on the antennae and the ultimate legs of <i>Scutigera coleoptrata</i> <i>Iulia Barutia (Institute for Zoology and Evolutionary Research, FSU)</i> <u>Abstract</u> page 16
16:20	8 - The co-option of spruce defensive stilbenes by a bark beetle symbiotic fungus against a natural entomopathogenic fungus <i>Baoyu Hu (Department of Biochemistry, MPI-CE)</i> <u>Abstract</u> page 17
16:35	9 - Experimental <i>Sodalis</i> infection eliminates ancient symbiont in a grain pest beetle <i>Ronja Krüsemer (Department of Insect Symbiosis, MPI-CE)</i> <u>Abstract</u> page 18
16:50	End
	Travel by regional train from Dornburg/Saale to Jena RB 25 to Saalfeld leaves Dornburg/Saale at 17:29 (arrives at Jena-Zwätzen at 17:35, Jena Saalbahnhof at 17:38, and Jena Paradies at 17:41)

April 3rd, 2025

	Travel by regional train from Jena Paradies to Dornburg/Saale RB 25 to Halle/Saale leaves at 8:15 a.m. in Jena Paradies (Jena Saalbahnhof 8:17, Jena-Zwätzen 8:20) and arrives in Dornburg at 8:27 a.m.
09:15	Plenary Lecture 2 (Großer Kaisersaal) "Exploring, elucidating and engineering complex flavonoid glycoside biosynthesis" <i>Sandra Irmisch, University of Leiden, The Netherlands</i> <u>Abstract</u> page 10
	Chair: Tobias Köllner
10:00	Coffee break (Rittersaal)

3 rd talk session	
	Chair: Yelyzaveta Zhyr
10:30	10 - Natural variation in defensive chemistry in <i>Lagria villosa</i> and its link to their symbiotic community <i>Johann de Beer (Department of Insect Symbiosis, MPI-CE)</i> <u>Abstract</u> page 19
10:45	11 - Facultative aphid endosymbionts can alter the host plant metabolome <i>Surhud Sant (Department of Biochemistry, MPI-CE)</i> <u>Abstract</u> page 20
11:00	12 - Explain yourself: Adapting SIRIUS fragmentation trees to GC-EI-MS by learning fragmentation behavior from the data Jonas Emmert Chair of Bioinformatics, FSU) Abstract page 21
11:15	13 - Development of single-cell mass spectrometry method for the study of the medicinal plant <i>Catharanthus roseus</i> <i>Ahn H. Vu (Department of Natural Product Biosynthesis, MPI-CE)</i> <u>Abstract</u> page 22
11:30	14 - Spreading dynamics in clonal raider ant interaction networks <i>Sarah Rogoz (Lise Meitner Group Social Behaviour, MPI-CE)</i> <u>Abstract</u> page 23
11:45	15 - Hawkmoths can smell with grooming organs on their legs Ahmed R. Ismaieel (Department of Evolutionary Neuroethology, MPI-CE) Abstract page 24
12:00	Lunch break (Rittersaal)
2 nd poster session (even numbers) Chair: Claudia Voelckel	
13:00	Bird's eye-view of 2023 PhDNet Survey responses for MPI-CE Bhoomika Bhat
13:30	One minute poster talks (2,4,6,8,10,12,14,16,18,20) (Großer Kaisersaal)
13:45	Poster session (Kleiner Kaisersaal & Flur)
15:15	Final Remarks All jury members submit their scores and rankings (hand to Anja or Claudia)
	Travel by regional train from Dornburg/Saale to Jena Paradies RB 25 to Halle/Saale leaves at 15:31 or 16:31 in Dornburg/Saale (Jena-Zwätzen 15:37/16:37, Jena Saalbahnhof) and arrives in Jena Paradies at 15:43/16:43).

PhD meeting (MPI for Chemical Ecology)	
18:00	Dinner (Cafeteria)
18:30	Introduction PhD representatives (A1.011 + A1.009) PhD survey discussion among doctoral researchers
19:00	Award ceremony Sarah O'Connor

- 1. Engineering of insect juvenile hormone III biosynthesis in the plant *Nicotiana benthamiana Angeliki Stathaki (Department of Natural Product Biochemistry, MPI-CE)*, <u>Abstract</u> page 26
- RNA-Guided Plant Protection for the Control of Pathogens Causing "Syndrome Basses Richesses" (SBR) Jonas Nötzold (FSU), Abstract page 27
- 3. Plant cell culture in the single cell era Sönke Beewen (Department of Natural Product Biosynthesis, MPI-CE), <u>Abstract</u> page 28
- Ozone Disrupts Social Behavior in Ooceraea biroi: A Markerless Multi-Animal Tracking Approach Eduardo Briceño-Aguilar (Department of Evolutionary Neuroethology, MPI-CE), <u>Abstract</u> page 29
- 5. Small and strict or large and loose? Symbioses in false click beetles *Jürgen Wierz (Department of Insect Symbiosis, MPI-CE)*, <u>Abstract</u> page 30
- 6. Multi-Omics Guided Discovery of Quinine Biosynthetic Genes Tingan Zhou (Department of Natural Product Biosynthesis, MPI-CE), Abstract page 31
- 7. The costs and benefits of chemical defense in the horseradish flea beetle *Phyllotreta armoraciae* (Coleoptera: Chrysomelidae) *Clara Boeninger (Plant-Biotic Interactions Group, IGZ)*, Abstract page 32
- 8. Too many cooks: The numerous symbionts of the plant disease vector *Pentastiridius leporinus Fortesa Rama (Department of Insect Symbiosis, MPI-CE)* <u>Abstract</u> page 33
- Beyond flight: exploring the olfactory role of *Manduca sexta* wings *Ahmed R. Ismaieel (Department of Evolutionary Neuroethology, MPI-CE)* <u>Abstract</u> page 34
- 10. Mechanism of metabolic regulation in grain pest beetle-endosymbiont interaction Soumi Bhattacharyya (Department of Insect Symbiosis, MPI-CE), Abstract page 35
- 11. *Arabidopsis thaliana* vtc2-1 mutant reduces aphid survival via metabolic shifts *Jessil Ann Pajar (IGZ)*, <u>Abstract</u> page 36
- SaxA-mediated degradation of antimicrobial 4MSOB-ITC functions as a public good for a leaf bacterial community *Kerstin Unger (Cluster of Excellence, FSU), Abstract page 37*
- Body temperature differences between green and brown grasshoppers do not result from thermal physiology or thermal preferences *Lilian Cabon (Population Ecology Group, FSU), <u>Abstract</u> page 38*
- How to befriend your enemy: Deciphering steps of mutualism establishment in a beetleendosymbiont system Sreyashi Mandal (Department of Insect Symbiosis, MPI-CE), <u>Abstract</u> page 39
- 15. Dimerization of bisindole alkaloids in Madagascar periwinkle *C. roseus* Susan Schlüter (Department of Natural Product Biosynthesis, MPI-CE), <u>Abstract</u> page 40
- Roles of volatile organic compounds in tri-trophic interaction along elevational gradient of Salix species Shristee Panthee (IGZ), Abstract page 41
- 17. Molecular regulation and ecological consequences of chemodiversity in *Solanum dulcamara Judit Valeria Mendoza Servín (Plant-Biotic Interactions Group, IGZ),* <u>Abstract</u> page 42

- Regulation of the supply of the universal precursor molecules of terpenoid biosynthesis, isopentenyl diphosphate and dimethylallyl diphosphate, by the MEP and MVA pathway in two woody plant species *Jia Zhang (Department of Biochemistry, MPI-CE), Abstract* page 43
- The formation of iridoid trail pheromones in ants Maithili Datta (Department of Natural Product Biosynthesis, MPI-CE) Abstract page 44
- 20. The role of recurrent long- and short-range connections in experience-dependent modulation in Drosophila

Sayantani Biswas (Research Group Olfactory Coding, MPI-CE) Abstract page 45

Talks



Plenary Lecture 1

Glowing belowground: What are the Ecological Functions of Bacterial Bioluminescence in Soil Ecosystems?

Arthur Muller¹, Patricia Morales-Montero¹, Alexandre Hiltmann¹, Ricardo A. R. Machado¹

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Apart from its entomopathogenic abilities, an intriguing biological trait of the bacterial genus Photorhabdus is the production of bioluminescence. Bioluminescence is the chemical production and emission of light by living organisms. This trait has evolved multiple independent times and occurs in more than eight hundred genera across the tree of life. While bioluminescence is well-studied in aquatic ecosystems, less is known about its ecological and evolutionary significance in terrestrial ecosystems, and almost nothing is known about the role of bioluminescence in belowground ecosystems. My group uses bioluminescent *Photorhabdus* bacterial symbionts as a model to understand the biological relevance of bioluminescence in the soil. Photorhabdus symbionts live in association with Heterorhabditis entomopathogenic nematodes. These nematodes penetrate soil-dwelling insects, move towards the insect hemocoel and release their Photorhabdus bacterial symbionts. Following the infection, Photorhabdus bacteria reproduce, produce toxins and immune suppressors that kill the insect prey. Nematodes then feed on bacteria-digested insect tissues, and reproduce inside the insect cadaver before emerging as infective juveniles to search for a new host. During the colonization process, Photorhabdus bacteria produce bioluminescence, which results in a characteristic glow of the infected insect cadavers. How this type of bioluminescence impacts the behaviour, performance, and physiology of other soil-dwelling organisms including entomopathogenic nematodes, plants, and predatory and scavenging insects, remains unknown. During my talk, I will present our findings in this context and will show that this unique bacterial trait is a powerful regulator of multi-trophic interactions in soil ecosystems.

Plenary Lecture 2

Exploring, elucidating and engineering complex flavonoid glycoside biosynthesis

Filipe Cruz¹, Charlotte Hijmans¹, Sophia Karon¹, Lars Kruse², Martijn Verkuilen¹, Klara Schmidt¹, Joerg Bohlmann², <u>Sandra Irmisch¹</u>

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Plants produce a large diversity of small molecules, called specialized metabolites which are an indispensable source and inspiration for medicinal drugs. However, valuable specialized metabolites are often rare in nature and due to their structural complexity not easily accessible through chemical synthesis. Thus, finding ways to discover new specialized metabolites and subsequently achieve their production to enable drug testing and development is a fundamental challenge. In my lab we combine metabolite discovery with pathway elucidation and engineering to obtain, diversify and test new metabolites which are otherwise difficult to produce.

In our recent work we elucidated the biosynthesis of the complex acylated flavonoid glycoside montbretin A (MbA), a new and improved treatment option for type 2 diabetes. Currently, MbA supply poses a challenge for drug development and application but synthetic biology might offer a solution. We showed proof-of-concept for production of MbA in heterologous hosts by reconstructing the pathway in the plant host *Nicotiana benthamiana* and the microbial host *Saccharomyces cerevisiae*.

Ongoing work focuses on yield improvement for example using transporter engineering. Additionally, we are exploring the medical activity, distribution and biosynthesis of complex glycosides within the plant kingdom with the ultimate aim to diversify the chemical space of plant metabolites that can be tested for therapeutic function.

Cracking the genetic basis of green-brown polymorphism in meadow grasshopper (*Pseudochorthippus parallelus*)

Xinyi Cheng^{1,2}, Nikhil Singh², Octavio M. Palacios-Gimenez^{2,3}, Mahendra Varma^{1,2}, Chongyi Jiang², Holger Schielzeth^{1,2}

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Phenotypic polymorphism is the direct object on which nature selection acts. Green-brown color polymorphism presents wide distribution in grasshoppers and serves as an ideal model for studying the selection effect on evolution. However, due to the large genome of grasshoppers and the corresponding high cost of sequencing, research on their genetic basis is still limited. Here, we constructed a high-density linkage map out of 4369 SNPs from meadow grasshopper (*Pseudochorthippus parallelus*). Comparison with the genome assembly of a closely related species, *Gomphocerus sibiricus*, exhibits a high extent of synteny between *P. parallelus* linkage groups and *G. sibiricus* genome. Based on the linkage map, we applied quantitative trait locus (QTL) mapping for the color morphs of *P. parallelus* and discovered one peak with strong signal, which suggests that the green/brown coloration is related to a single locus or multiple linked loci with close physical distances. In the next step, we will highlight several candidate genes for green/brown determination within the *G. sibiricus* homologous region of the QTL peak. In conclusion, this study will release the first high-quality linkage map resource of *Pseudochorthippus*, reveal the potential regulatory mechanism underlying color polymorphism of grasshoppers, and provide new opportunities for interspecific comparisons and evolutionary history inferences.

Sources of variation in plant chemical diversity

Linh M. N. Nguyen^{1,2,3}, Jana Ebersbach^{1,4}, Diary Razafimandimby⁵, Jean-Yves Rasplus⁶, Henriette Uthe⁷, Radoniaina R. Rafaliarison⁸, Stefanie Döll^{1,2}, Yvonne Poeschl⁹, Kim Valenta¹⁰, Nicole M. van Dam^{1,2,11}, Omer Nevo 1,2 ¹ German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany ² Institute of Biodiversity, Friedrich Schiller University Jena, Jena, Germany ³ Max Planck Institute for Chemical Ecology, Jena, Germany ⁴ Department of Molecular Evolution and Plant Systematics and Herbarium (LZ), Leipzig University, Leipzig, Germany ⁵ Faculty of Sciences, Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar ⁶ CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, University of Montpellier, Montpellier, France ⁷ MetaCom Program Center, Leibniz Institute for Plant Biochemistry, Halle, Germany ⁸ Mad Dog Initiative. Akanin'ny Veterinera Akaikiniarivo, Antananarivo, Madagascar ⁹ Faculty of Natural Sciences III, Institute of Agricultural and Nutritional Sciences, Biometrics and Agroinformatics, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany ¹⁰ Department of Anthropology, University of Florida, USA ¹¹ Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Grossbeeren, Germany

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Plants, like many other organisms, produce a tremendous variety of secondary compounds that are crucial to interspecific and intraspecific interactions, as well as to adaptation to environmental changes. This chemical diversity has been attributed to multiple factors such as function (e.g., defense, pollinator attraction), tissue-specific needs, various constraints, and phylogenetic history. Yet the interplay between these multiple factors driving plant chemodiversity is still unclear, primarily because the vast majority of studies focused on a single organ- (mostly leaves), or when comparing different organs, focused on single taxa. As such, the interplay between function and phylogenetic effects remains unclear. Using a model system of wild figs from Madagascar, this study tests (a) the degree to which phylogeny explains variation in fruit and leaf chemodiversity (b) to what degree phytochemical diversity is explained by tissue-specific function. We applied an untargeted metabolomics approach to unripe fruits and leaves from eight species of wild figs (Ficus spp) sampled in a tropical rainforest in Madagascar. We characterized their chemical profiles using ultraperformance liquid chromatography-mass spectrometry and, using six genetic markers, reconstructed their phylogeny to understand the patterns of chemodiversity. We found a significant, but moderate phylogenetic effect in fruit chemodiversity, and no phylogenetic effect in leaf chemodiversity. Fruit and leaf metabolomes were more similar to the same tissue in other species than to the other tissue within the species. Our results indicate that, while phylogeny plays a role in plant chemodiversity, functional convergence of tissue-specific metabolites is a major driver of plant chemodiversity.

The chemical and sensory bases of social immunity in the clonal raider ant

Sandra Tretter¹, Veit Grabe², Silke Sachse³, Yuko Ulrich¹

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Social insects live in densely populated colonies, which increases the risk of disease outbreaks. As a result, they have developed collective strategies for disease defense known as 'social immunity.' For instance, social insects groom infectious fungal spores from each other's body surfaces to prevent infection. Social immunity depends on the ability to identify infected conspecifics, but the chemical and sensory bases of this detection remain poorly understood.

We aim to explore the mechanistic bases of social immunity using the queenless clonal raider ant *Ooceraea biroi,* a blind species that likely detects sick nestmates by olfactory cues. However, other modalities (e.g., gustation) might also play a role in detecting sickness-related cues. Our objectives are to 1) identify candidate olfactory "disease-related cues" by comprehensively screening the chemical profiles of infected ants, 2) characterize the behavioral responses of workers towards nestmates infected with different pathogens and using this response to functionally test candidate chemicals, and 3) investigate how "disease-related cues" are encoded in the brain through in vivo calcium imaging of the antennal lobe. Additionally, we will examine neuronal responses to pathogen odors and compounds, that are known to trigger social immunity behavior (e.g., ergosterol). Preliminary behavioral data indicates a response of non-infected nestmates towards infected individuals at specific time points following bacterial infection.

By combining chemical, neuronal, and behavioral analysis, we anticipate shedding light on the mechanistic bases of social immunity in insect societies.

The Not-So-Sweet Life of a Sweet Potato: Metabolic Changes Upon Mutualistic and Pathogenic Interactions

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Symbiotic relationships play an integral role in the functioning of the natural world. While some interactions can prove highly beneficial, others can be detrimental; however, organisms rarely experience these relationships in just one form. For plants, as sessile organisms, this means that they are constantly exposed to a variety of interactions - mutualistic, commensal, and parasitic. While this dynamic can present challenges, studying the interplay between these different types of relationships will provide a more natural context for understanding plant life. As a result, this knowledge could facilitate the development of more sustainable agricultural practices.

Sweet potato (*Ipomoea batatas* [L.] Lam) is one of the most widely cultivated crops in the world due to its nutritional value and resilience to current climatic threats. However, despite its growing importance in agriculture, *I. batatas* remains a relatively understudied species. There is a notable lack of research investigating the interactions between sweet potatoes and beneficial or pathogenic fungi, or the consequences when both occur concurrently. We aim to use advanced analytical and computational tools to characterize plant metabolomics during their simultaneous interactions with mycorrhizal (*Rhizophagus irregularis*) and necrotrophic (*Alternaria alternata*) fungi. This investigation includes the analysis of primary and secondary metabolites, volatiles, and root exudates to characterize metabolic changes in sweet potatoes both above and below ground.

NOT Everything Everywhere All at Once: How Interdependent Symbionts in a Beetle Work

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Many insects have successfully invaded unhospitable environments due to their close association with symbiotic microbes. These co-evolving partners provide, for example, rare nutrients and expand host ecological niches. Among them, several grain pest beetles across different families have adapted to dry conditions with poor nutrients thanks to their ancient endosymbionts which supply precursors of tyrosine, an aromatic amino acid essential for beetle cuticle formation. In the larger grain borer *Prostephanus truncatus*, an extreme case of symbiont genome reduction has led to the divergence of *Shikimatogenerans bostrichidophilus* into three interdependent strains, each contributing complementary metabolic functions.

Harnessing the power of integrated genomic and imaging approaches to gaining insight into complex insect-microbe interactions and their evolution, we aimed to investigate the mechanisms of cooperation among these symbionts Specifically, we examined how and which molecules they exchange and but also discovered spatiotemporal variations in the symbiont cell structures throughout the beetle's life. Given their inability to be cultured outside the host, we employed *in situ* hybridization techniques to target strain-specific molecules and combined fluorescent and electron microscopy in 2D and 3D for ultrastructure analyses.

Our genome analyses and imaging revealed that three cytologically distinct strains are essential for completing the shikimate pathway, requiring the exchange of macromolecules or proteins. Diet manipulation experiments further confirmed a functional nutrient supplementation to the host. Notably, dynamic structural changes and connections among symbiont cells are likely related to metabolic exchange and transovarial transmission of symbionts.

While lineage splitting of symbionts with reduced genome sizes has been reported in a few insects, no adaptive scenarios have been identified. Our results identified also physiological adaptations to keep this fragmented symbiosis functionally intact and thereby highlight evolutionary processes to counter ongoing genome erosion.

From genes to ecosystems: ecological role and agricultural potential of gramine

<u>Sara Leite Dias</u>¹, Paride Rizzo¹, Ajith Kochukulam Ravindran¹, Samuel Schuck², Fritz Forbang Peleke¹, Fabian Brendel¹, Galien Flückiger³, Grit Kunert³, Jędrzej Szymański¹, Matthew Agler², Andriy Kochevenko⁴, John C. D'Auria¹

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Gramine is an alkaloid found in barley and other members of the Poaceae that provides protection against insects and influences their palatability to ruminants. We recently resolved the biochemical basis for gramine formation from the amino acid tryptophan by identifying a gene cluster in barley containing two key genes encoding a previously reported N-methyltransferase as well as the AMI synthase (AMIS). The elucidation of the missing step in this biosynthetic pathway opens up new avenues for research and applications.

Beyond its genetic basis, we are also interested in the regulation of these genes. Therefore, to understand how this pathway is regulated, we are investigating cis-regulatory elements (CREs) and transcription factors (TFs) using deep learning models that predict gene expression, which we will correlate with metabolite accumulation.

Moreover, we aim to investigate the ecological implications of the presence of this alkaloid. How does gramine shape barley's interactions with herbivorous insects and bacterial communities?

We are exploring the role of gramine in plant-herbivore interactions, focusing on aphids—economically significant barley pests. The project aims to determine how gramine affects aphid performance and whether there are differences in susceptibility between the generalist (*Myzus persicae*) and specialist (*Rhopalosiphum padi*) aphids. Additionally, we are investigating how leaf-colonizing microorganisms, including opportunistic pathogenic bacteria, respond to the presence of gramine. Although plants appear healthy, their bacterial microbiomes may be shaped by gramine. Using 200 barley accessions with varying gramine levels, we employ amplicon sequencing to assess how the bacterial community assembles in response to this metabolite.

While identifying the genes responsible for gramine biosynthesis is a major step forward, it is only the beginning. By leveraging this knowledge, breeding programs can aim to reduce pesticide use and harness the biological activity of gramine to enhance barley resilience.

Heads or tails? Sensory equipment on the antennae and the ultimate legs of *Scutigera coleoptrata*

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The house centipede, Scutigera coleoptrata, is one of the fastest invertebrate predators. Endemic to the mediterranean region, this species is nowadays also common throughout central Europe and North America. Scutigeromorph centipedes are generally characterized by very long legs and antennae, covered with thousands of sensory structures. They are exceptionally fast and agile, and when hunting, they use their slender legs like lassos to immobilize prey before injecting their venom. Unique to centipedes is the functional transformation of the last pair of legs, called the ultimate legs, that show a considerable structural disparity and functional diversity. In scutigeromorphs, the ultimate legs are not involved in locomotion or prey capture and they are much longer than the locomotory legs. In fact, the length of the ultimate legs, as well as their position and kinematics highly resemble that of the antennae. Due to this resemblance, it is assumed that the ultimate legs have a predominantly sensory function, similar to the antennae. As a first step to test this hypothesis, we used SEM and TEM imaging to analyze the types of sensilla (as well as their distribution and anatomy) found on both the antennae and the ultimate legs, and compared these with sensilla on the locomotory legs. While some sensilla are specific to a certain type of appendage, there is a striking resemblance between the number and distribution certain sensilla on the antennae and the ultimate legs. Moreover, the locomotory legs show a distinct pattern of distribution of the sensilla, in contrast to both the antennae and the ultimate legs. Thus, the results of our morphological experiments are in favor of the hypothesis of a pronounced sensory function of the ultimate legs. Moreover, our data suggests that the beak-like sensilla, a sensillar type found exclusively in scutigeromorph centipedes (on all appendage types) might serve an olfactory function. This is the first morphological evidence pointing towards olfaction in this group of centipedes, and unraveling the mechanism behind it might shed light on the evolution of olfactory systems in terrestrial arthropods. Further on, neuroanatomical and electrophysiological experiments will follow in order to confirm the functional transformation of the ultimate legs, as well as to further test the possible olfactory function of beak-like sensilla.

The co-option of spruce defensive stilbenes by a bark beetle symbiotic fungus against a natural entomopathogenic fungus

Baoyu Hu¹, Jonathan Gershenzon¹, Ruo Sun¹ ¹Department of Biochemistry, Max-Planck Institute for Chemical Ecology

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The attack of the bark beetle *lps typographus* and its associated fungi has resulted in extensive destruction of spruce trees across the world. To cope with the colonization of insects and infection of pathogenic fungi in its trunk, Norway spruce (*Picea abies*) has developed integrated chemical defenses. Stilbenes in the bark of spruce trees are phenolic compounds that are utilized as important defenses. However, the mechanism by which bark beetles and their fungal associates resist spruce defensive stilbenes remains unclear. In this study, we investigated the metabolism of spruce stilbenes by one of major bark beetle fungal associates, *Endoconidiophora polonica*. We found that *E. polonica* converts stilbene glycosides into aglycones, followed by methylation via catalysis of an O-methyltransferase. Next, the biological activities of the methylated products against other organisms present in bark beetle galleries were tested. The methylated stilbene metabolites formed by *E. polonica* were found to significantly inhibit growth of the bark beetle entomopathogenic fungus *Beauveria bassiana*, which could be benefitical to the beetle. In future work, the impact of the stilbene products formed by *E. polonica* will be explored on the survival of bark beetles and other organisms in the bark beetle ecosystem.

Experimental Sodalis infection eliminates ancient symbiont in a grain pest beetle

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Intracellular symbioses with microorganisms have proven to be a critical factor in insect diversification. In many cases these mutualistic associations are obligate and host and microbial symbiont have become metabolically dependent on each other via coevolution. Despite the obligate nature of many intracellular insect symbioses, ancient symbionts can be replaced by other microbes. The exact mode of action, however, remains unclear since the intractability of most naturally occurring symbiotic associations has hampered the experimental investigation of these replacement events. To address the mechanisms that drive symbiont replacements, as well as symbiosis establishment and maintenance, tractable study systems allowing for the manipulation of existing symbioses are needed. Here, we describe our attempt to establish such a system in Oryzaephilus surinamensis, a grain pest beetle that natively houses the non-obligate, tyrosine-provisioning symbiont Shikimatogenerans silvanidophilus. We introduced a free-living relative of many insect symbionts, Sodalis praecaptivus, to the host and investigated the new symbiont's effect on host and native symbiont. S. praecaptivus established systemic infections in the beetle host and was vertically transmitted with high fidelity. The new symbiont was mildly pathogenic, reducing beetle lifespan as well as reproductive fitness and impairing cuticle melanization in the first offspring generation. Strikingly, the presence of S. praecaptivus also negatively affected the beetle's native symbiont: Colocalization of Sodalis with Shikimatogenerans in the bacteriomes led to aberrant morphology of the ancient symbiont and ultimately resulted in the rapid elimination of Shikimatogenerans after three host generations. We also observed the inability of the native symbiont to modulate its gene expression in response to the invading bacterium, leaving it prone to being outcompeted by Sodalis. We hypothesize that the rapid elimination of the ancient symbiont recapitulates the first step in an experimental symbiont replacement, paving the way for the establishment of a novel symbiosis with S. praecaptivus.

Natural variation in defensive chemistry in *Lagria villosa* and its link to their symbiotic community

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In nature, the prevalence of insect antagonists is dynamic in space and time. Defensive symbiosis in insects can protect the host's life stages against predators, parasites and pathogens. The presence of a complex microbial community producing diverse secondary metabolites may enable the host to adjust its defences to cope with changes in antagonist communities. In the beetle species Lagria villosa (Coleoptera, Tenebrionidae), vertically transmitted bacterial symbionts (chiefly Burkholderia) protect eggs and larvae against fungal pathogens by producing antimicrobial metabolites. The most prominent bioactive compound is the antifungal polyketide lagriamide, produced by the dominant Burkholderia gladioli strain Lv-StB. Other members of the microbial community, such as Luteibacter and Variovorax also show potential for the production of bioactive compounds. Given the diversity of microorganisms associated with L. villosa in the field, this study sought to understand how the variability of secondary metabolites is linked to this diverse symbiotic community in the face of variable antagonistic interactions. Different life stages of L. villosa beetles were collected from soybean fields in Brazil. In each individual, the presence of bacterial secondary metabolites was elucidated through HPLC-MS/MS, and their symbiotic community was characterised through 16s rRNA sequencing. Interestingly, by far the most prevalent secondary metabolite elucidated was lagriamide, which occurred in highly variable concentrations that likely correlate with the abundance of B. gladioli Lv-StB and the composition of the remaining bacterial community. Ultimately, this project will resolve how the chemical variation associated with L. villosa relates to their bacterial community, providing interesting insights into the dynamics and relevance of defensive symbioses under natural conditions.

Facultative aphid endosymbionts can alter the host plant metabolome

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The pea aphid, *Acyrthosiphon pisum*, comprises at least 15 genetically distinct biotypes, each specialized on a limited range of legume hosts. However, all biotypes are able to perform well on the universal host, *Vicia faba*. All pea aphids contain the obligate endosymbiont *Buchnera aphidicola*, which provides essential amino acids to the aphids, and some aphids also carry one or more facultative endosymbionts. The *Trifolium*-adapted pea aphid biotype is predominantly associated with the facultative endosymbiont *Regiella insecticola*. While *R. insecticola* confers protection against entomopathogenic fungi and parasitoids, its prevalence in the *Trifolium* biotype remains unexplained.

From previous experiments, we know that *R. insecticola* has little effect on aphid fitness when aphids feed on their native host plant, *Trifolium pratense*. However, when feeding on *V. faba*, aphids carrying *R. insecticola* showed reduced fecundity and increased mortality. We hypothesized that this might be due to enhanced plant defense responses in *V. faba*. However, while aphid feeding induced salicylic acid (SA) and SA-Glu accumulation, other phytohormones (ABA, JA-IIe, OH-JA-IIe, COOH-JA-IIe) remained unchanged and endosymbiont presence did not significantly affect their levels.

However, metabolomic analysis revealed distinct plant responses to aphid feeding and its endosymbiont composition. In particular flavonoids and associated glycosylated compounds changed depending on whether the feeding aphids contained *R. insecticola* or not. Given the antimicrobial properties of flavonoids, we hypothesized that endosymbionts could be affected, and examined endosymbiont titers in the aphid at different developmental stages. *R. insecticola* consistently showed higher titers than *B. aphidicola* levels significantly reduced in later aphid developmental stages when *R. insecticola* was present. Lower *B. aphidicola* titer correlated with lower levels of essential amino acids (Met, Ile, Leu, Phe, Trp), which could consequently lead to reduced aphid performance.

Our findings suggest that the reduced performance of *R. insecticola*-carrying aphids on *V. faba* may result from a combination of host plant metabolic changes and changes in endosymbiont titers.

Explain yourself: Adapting SIRIUS fragmentation trees to GC-EI-MS by learning fragmentation behavior from the data

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Tandem mass spectrometry (MS/MS) with electrospray ionization (ESI) is a cornerstone of untargeted metabolomics, but interpreting fragmentation spectra remains a major challenge. Methods like SIRIUS propose molecular formulas by modeling fragmentation reactions as fragmentation trees, assigning a molecular formula as the root and formula-annotated fragments and losses as nodes and edges of the tree, respectively. Trees are then scored based on multiple factors, and the highest-scoring tree determines the best fitting molecular formula. However, scoring fragmentation trees requires an understanding of which fragments and losses are likely or improbable - a task that becomes increasingly complex as peak mass rises and multiple molecular formulas become plausible explanations.

In this presentation, we revisit the methodology for inferring missing prior knowledge directly from data, with a focus on adapting the SIRIUS fragmentation trees to gas chromatography coupled electron ionization mass spectrometry (GC-EI-MS). We begin by defining a set of criteria for evaluating the plausibility of molecular formulas and introduce empirical distributions of observed mass and intensity values. The framework is then refined through Maximum A Posteriori estimation, an iterative process in which posterior knowledge about common fragments and losses, derived from the data, is incorporated as prior knowledge for subsequent iterations. The convergence of this adaptive process yields a final model for fragmentation tree scoring in GC-EI-MS. We conclude by presenting initial evaluations of molecular formula retrieval for GC-EI-MS data, benchmarking performance against the well-established fragmentation trees used in ESI-MS/MS.

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

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Plants synthesize valuable natural products that are widely used in the pharmaceutical, agrichemical, flavor, and fragrance industries. Remarkably, these structurally complex molecules are not evenly distributed throughout plant tissues; instead, they are synthesized and stored in specific cell types. Mapping the precise location of these metabolites at the cellular level would greatly facilitate our understanding of both their biosynthesis and function. However, the detection, identification, and quantification of metabolites in single cells, especially in plants, have long posed challenges. In this study, we demonstrate that we are able to definitively identify and quantify different molecules from four classes of natural products in individual cells of leaf, root, and petal tissues of the medicinal plant *Catharanthus roseus* using an in-house developed single-cell mass spectrometry technique. This method provides high resolution insights into the spatial partitioning of plant natural product biosynthesis at a cell-specific level.

Spreading dynamics in clonal raider ant interaction networks

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The structure of social interaction networks is theoretically predicted to affect spreading dynamics in social groups, including disease spread. However, few systems afford the degree of experimental control required to empirically test the link between network structure and pathogen spread. The problem alleviated in the clonal raider ant, a queenless social insect in which workers reproduce synchronously and clonally. This unusual biology makes it possible to precisely control and replicate several colony traits that are expected to affect social network structure, including colony size, genetic and demographic structure. Here, we investigate the effect of group composition on network structure, and in turn, predicted disease spread.

Specifically, we use automated tracking to measure social networks in clonal raider ant colonies with controlled variation in size, age structure and genetic structure. We predict that these factors induce predictable and measurable differences in network structure between colonies, and ask how these differences in turn affect predicted disease spread. This work provides the first step towards experimentally testing predictions from theoretical epidemiology and bridging the gap between simulated and real-world epidemics.

Hawkmoths can smell with grooming organs on their legs

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Insects rely on specialized sensory organs for olfaction, traditionally attributed to their antennae. However, our study reveals that the epiphysis, a grooming organ on the forelegs of *Manduca sexta*, may also play an olfactory role. Using scanning electron microscopy (SEM), we identified two types of sensilla on the epiphysis: one lacking finger-like cuticular processes and another with 2–6 processes. Both sensillum types have wall pores, indicating olfactory function. To explore the molecular basis of this putative sensory capacity, we performed gene expression analysis using the NanoString technology. We detected 55 odorant receptors (ORs), 22 ionotropic receptors (IRs), and 33 gustatory receptors (GRs) expressed in the epiphysis. Electrophysiological recordings from the whole epiphysis and from single sensilla on the epiphysis revealed that both male and female epiphyses respond to various odorants, particularly acids and pyrrolidine, and to ecologically relevant odor mixtures such as headspace from flowers of nectar sources, leaves of host plants and an extract from the female pheromone gland. Finally, wind tunnel experiments demonstrated that epiphysis-ablated gravid females were impaired in their ability to locate a host plant for oviposition. These findings suggest that the epiphysis plays a role in the olfactory-guided behavior of *M. sexta*, expanding our understanding of olfactory tissues in insects.



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Engineering of insect juvenile hormone III biosynthesis in the plant Nicotiana benthamiana

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Juvenile hormones (JHs) are farnesoic acid-derived sesquiterpenoids that play a crucial role in regulating various developmental processes in insects. Based on these reported biological activities, JHs and their synthetic analogs have been utilized as insecticides with significant commercial success over the past years. Here we describe the engineering of the JH pathway of the yellow fever mosquito (*Aedes aegypti*) by transient gene expression in the plant *Nicotiana benthamiana*. This approach led to the successful production of JH III in *N. benthamiana* leaves at a concentration of ca. 10 µg/g fresh weight. The co-expression of a feedback-insensitive version of 3-hydroxy-3-methylglutaryl coenzyme A reductase from *Arabidopsis thaliana* further increased the titer eight-fold from 10 to 80 µg/g fresh weight. Our efforts also revealed that the rich endogenous metabolic background of *N. benthamiana* can generate farnesoic acid, a key precursor to JH III, and thus, only 3 genes need to be expressed to provide high titers of this compound. Our study demonstrates the production of high titers of JH III in *N. benthamiana* the production of high titers of JH III in *N. benthamiana* the production of high titers of JH III in *N. benthamiana* the production of high titers of JH III in *N. benthamiana* the production of high titers of JH III in *N. benthamiana* the production of high titers of JH III in *N. benthamiana* the production of high titers of JH III in *N. benthamiana* to heterologous expression of insect JH biosynthetic genes.

RNA-Guided Plant Protection for the Control of Pathogens Causing "Syndrome Basses Richesses" (SBR)

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The phloem-sucking planthopper *Pentastiridius leporinus* is rapidly expanding at a rate of approximately 25 km per year and will probably have infested all 60,000 hectares of sugar beet cultivation area in Bavaria in the near future. *P. leporinus* transmits *Candidatus Arsenophonus phytopathogenicus* as well as *Candidatus Phytoplasma solani*. The pathogens are responsible for the *Syndrome Basses Richesses* (SBR), leading to significant losses in sugar content and biomass yield. In order to prevent the loss of sugar beet as a mainstay of the rural economy, the development of effective control measures is required. RNA-based plant protection concepts show particular potential compared to conventional plant protection. RNA active components used are highly effective and can be targeted at both the vector insect and the bacteria due to their sequence-dependent mode of action.

This project aims to develop innovative, highly effective, and targeted RNA-based plant protection strategies against P. leporinus and its associated pathogens. The research focuses on: (1) the design and validation of RNA-based molecules (dsRNA and antisense circRNAs) to selectively silence kev target aenes in Ρ. leporinus and bacterial pathogens. (2) the development of phloem-mobile RNA formulations for effective field application, (3) the evaluation of RNA uptake, stability, and systemic movement in plants and insect vectors, and (4) large-scale field trials to assess efficacy under real agricultural conditions.

The project is a collaborative effort among leading institutions in RNA biotechnology, phytopathology, entomology, bacteriology, and plant physiology. This interdisciplinary approach integrates cutting-edge molecular research with applied agricultural solutions, paving the way for sustainable and environmentally friendly crop protection strategies.

Plant cell culture in the single cell era

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Plant-derived natural products serve as a crucial source of widely used medicines. However, their natural abundance in plant tissues is often extremely low, necessitating the harvesting of large quantities of plant material-sometimes from endemic or endangered species-to obtain only a few grams of the desired compound. The chemical complexity of these molecules, such as the anticancer drug vinblastine, makes their total chemical synthesis economically unfeasible. Plant tissue culture, leveraging naturally occurring callus material, presents a promising alternative for sustainable and scalable production of these valuable compounds. By cultivating plant-derived tissues under controlled conditions in large-scale batch cultures, tissue culture offers the potential to improve both yield and costeffectiveness, Despite significant research efforts in the 1980s, widespread application of plant tissue culture has remained challenging due to difficulties in its maintenance and regulation, largely stemming from a limited understanding of the fundamental developmental and organizational processes governing these tissues. Recent advancements in single-cell analysis now provide powerful tools to address these challenges. By employing single-cell transcriptomics, we can systematically classify cell types within callus tissues, while single-cell metabolomics enables us to map the distribution of biosynthetic pathways across these cell populations. This integrative approach allows us to construct a detailed molecular atlas of callus cultures, identify differentially regulated genes, and ultimately optimize biosynthetic flux toward the enhanced production of high-value medicinal compounds.

Ozone Disrupts Social Behavior in *Ooceraea biroi*: A Markerless Multi-Animal Tracking Approach

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Eusocial insects, including ants, rely heavily on cuticular hydrocarbons (CHCs) for nestmate recognition and social cohesion. However, these chemically mediated interactions are threatened by oxidative pollutants such as ozone, which could degrade key hydrocarbons involved in communication. Here, we investigate how prolonged ozone exposure affects social behavior in Ooceraea biroi, a clonal ant species that operates without a queen. Specifically, we assess whether ozone alters colony-level recognition of larvae and overall activity patterns. We exposed small functional colonies constituted of 10 workers and 8 larvae to either ambient air or 100 ppb ozone continuously for 12 days. Each day, we recorded 20-minute videos under controlled conditions (25 °C, 70% humidity) at a fixed time (1 PM) to monitor behavioral changes. Movement trajectories and activity were analyzed using TRex, a markerless multi-animal tracking system, and time spent near larvae was quantified using Euclidean distance measures. Preliminary observations suggest a tendency for reduced time near larvae and lower overall activity in ozone-exposed colonies, consistent with the hypothesis that ozone disrupts CHCmediated recognition. These findings highlight the potential for environmental pollutants to interfere with insect social behavior, with implications for colony functioning and survival. Through automated markerless tracking, we provide a reproducible fast and easy approach to understanding how anthropogenic byproducts disrupt brood care, with potential ecological consequences.

Small and strict or large and loose? Symbioses in false click beetles

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Symbiotic relationships with microbes are widespread in insects, traversing a broad spectrum of reciprocal dependency, intimacy, duration, and cooperation. Many insect taxa are only able to establish in their ecological niche with the help of bacterial partners. The false click beetles (Coleoptera, Throscidae) are a small, but globally distributed family of beetles that live in leaf litter and decaying wood and were previously reported to harbor intracellular symbionts in specialized bacteriomes. In this study, we investigated three of the four extant genera of Throscidae beetles for their symbiotic microbes and provide insights into host-symbiont interactions based on symbiont genomes and localization. The ancient Shikimatogenerans symbiont with a highly eroded genome of ~150 kbp and ~12% GC was present in all examined taxa, probably supplying tyrosine precursors for cuticle biosynthesis as its only contribution to the host. Beyond this, several secondary symbionts from the Enterobacterales (e.g. Sodalis and Symbiodolus) and Flavobacteriales with variable tissue tropism, genome sizes, and encoded metabolic capabilities were present, suggesting multiple symbiont acquisition events. Their inferred roles based on genome content and localization range from parasitic by manipulating host reproduction, to mutualistic, supplying multiple amino acids and cofactors. Thus, beyond deepening our understanding of tyrosine-supplementing symbionts across a broad range of beetle families with different ecologies, the Throscidae provide insights into a dynamic evolutionary history with multiple cooccurring symbionts and expand our view on multipartite symbiotic interactions in insects.

Keywords: Coleoptera, Throscidae, tyrosine supplementation, Shikimatogenerans, genome erosion, host symbiont coevolution, cocladogenesis, dual symbiosis, bacteriome, symbiont replacement

Multi-Omics Guided Discovery of Quinine Biosynthetic Genes

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Quinine, first isolated in 1820 from the bark of *Cinchona* trees, is a crucial anti-malarial drug and a historically significant natural product. Despite the successful total synthesis of quinine decades ago, its biosynthetic pathway remains largely unknown. As part of our ongoing efforts to uncover the molecular basis of quinine biosynthesis in *Cinchona* plants, we report the discovery of four key enzymes using a combination of transcriptomics (single-nucleus and bulk-tissue RNA sequencing), metabolomics, and feeding experiments with isotopically labeled compounds. The first identified enzyme is a cytochrome P450 that acts on cinchonaminal – a central intermediate – and on its methoxylated analog, converting their indole scaffolds into keto-quinoline structures, like the pre-quinine alkaloid quininone. These keto-quinolines are subsequently metabolized by an aldo-keto reductase, producing quinine and its analogs. The third discovered enzyme is a strictosidine glucosidase, a previously missing enzyme involved in the early-stage biosynthesis of corynantheol. The final enzyme, an oxoglutarate-dependent dioxygenase, is discovered following the identification of a novel quaternary amine intermediate and is demonstrated to function as a cinchonaminal synthetase. These findings pave the way for the identification of the remaining enzymes required to convert corynantheol into the quaternary amine intermediate, bringing us closer to the fully elucidation of the quinine biosynthetic pathway.

The costs and benefits of chemical defense in the horseradish flea beetle *Phyllotreta armoraciae* (Coleoptera: Chrysomelidae)

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Chemical defense against herbivores is very common in plants and consequently many insects have adapted to the specialized metabolites. The sequestration of toxic compounds or precursors of toxic products by insects is one way of adaptation which comes along with physiological costs and has a strong influence on tri-trophic interactions. The horseradish flea beetle, Phyllotreta armoraciae, possesses an activated defense system consisting of sequestered glucosinolates and endogenous myrosinase enzymes to produce toxic hydrolysis products such as isothiocyanates. While all life stages of P. armoraciae contain high amounts of sequestered glucosinolates, high levels of myrosinase activity were only detected in larvae and adults, suggesting that only these two life stages are chemically protected. Here we want to investigate the physiological costs and benefits of the flea beetle's chemical defense. To do so, we will generate myrosinase knock-out lines using the CRISPR/Cas9 system. In this way we can first determine the potential physiological costs that come along with myrosinase expression and activity by comparing performance and fitness measures in myrosinase-knock-out and wildtype P. armoraciae beetles. This will include observations of life history traits like developmental time, larval growth and reproduction efficiency. To assess the benefits of this chemical defense, we will use the wildtype and knock-out lines to investigate whether and to what extent the beetle myrosinases contribute to the metabolism of the sequestered glucosinolates. The endogenous turn-over of glucosinolates, that has been observed independent of predator attack, might also provide resistance against pathogens and nematodes, as isothiocyanates have antimicrobial and nematicidal properties. Therefore, we will investigate whether and to what extent P. armoraciae beetles are chemically protected against entomopathogenic fungi and nematodes, which are used as biocontrol agents against insect pests.

Too many cooks: The numerous symbionts of the plant disease vector *Pentastiridius leporinus*

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In Central Europe, *Pentastiridius leporinus* (Hempiptera: Cixiidae) is a major vector of the two causative agents of the Syndrome Basses Richesses (SBR) disease in both sugar beet and potato. Identified as *'Candidatus Arsenophonus phytopathogenicus'* and stolbur phytoplasma *'Candidatus Phytoplasma solani'*, the disease mechanisms of these bacterial plant pathogens are however unknown. In addition to the two aforementioned plant pathogenic bacteria, the insect host harbors three putative primary symbionts and two additional bacterial species of unknown function, thus creating a multipartite system of unusual complexity. In this intertwined system of many moving parts, we aim to disentangle the roles of the primary and secondary symbionts as well as the disease mechanisms of the vectored phytopathogens. Through dual RNAseq analysis and perturbation of symbiotic associations using antibiotics, RNAi and entomopathogenic agents, changes in host physiology, bacterial titers and disease progression will be analyzed. Additionally, we will investigate the evidently rapid host expansion of the vector as well as defense mechanisms within host plants through metagenome analysis of in-field bacterial associations, microscopy-based surveillance of disease progression and culturing of pathogens with subsequent genetic manipulation.

Beyond flight: exploring the olfactory role of *Manduca sexta* wings

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The wings of some insect species are known to have a chemosensory function. In the hawkmoth Manduca sexta, however, a possible olfactory role of the wings has not been studied previously. Here, we investigated the distribution and function of putative olfactory sensilla on the wings of *M. sexta* using scanning electron microscopy (SEM), gene expression analysis, electrophysiology, and behavioral assays. SEM revealed 21: 31 porous olfactory sensilla localized exclusively on the wing margin, with higher numbers on hindwings compared to forewings, and in females versus males. Gene expression analysis identified 11 odorant receptors (ORs), 2 ionotropic receptors (IRs), and 2 gustatory receptors (GRs) expressed in the wing margin of both sexes. Electrophysiological recordings using monomolecular odorants and headspace of ecologically relevant sources demonstrated that only the amine pyrrolidine elicited responses from wing sensilla. Behavioral experiments showed that surgical removal of wing margins impaired foraging efficiency in both sexes: operated females took longer to locate food sources than controls, while males exhibited longer flower contact than controls. However, mating and oviposition behaviors remained unaffected. These findings reveal that wing margin sensilla in *M. sexta* contribute to olfactory-guided foraging but not to oviposition and reproductive behaviors, highlighting a novel role for wings in chemosensory ecology. The sex-specific differences in sensilla numbers and behavioral outcomes suggest potential evolutionary adaptations linked to sex-specific resource acquisition strategies. This study expands our understanding of non-antennal olfactory systems in Lepidoptera and their ecological implications.

Mechanism of metabolic regulation in grain pest beetleendosymbiont interaction

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Symbiotic associations of insects with microorganisms represent a pivotal role in the huge evolutionary success of insects. The mutualistic associations with intracellular symbiotic bacteria, commonly referred to as endosymbionts, plays a crucial role in providing insects with essential metabolic components absent in their dietary intake. Tyrosine is the primary metabolite requisite for the synthesis of a hard and melanized cuticular exoskeleton; the primary line of defense in the beetles. However, beetles need to acquire tyrosine either from their diet given the inherent inability of all animals to synthesize tyrosine on their own. Insects repeatedly evolved specialized cells and entire organs to harbor endosymbionts, called bacteriocytes and bacteriomes. These serve as biosynthetic factories to supplement the host with tyrosine precursors in nutritionally unbalanced environments. While the support of cuticle formation represents an ecological benefit, the metabolic investment into symbiont growth incurs also costs for the host. Thus, host insects need to balance the cost and benefits of maintaining the endosymbionts, aligning their investment with physiological demands. However, the molecular mechanisms underlying such host control are poorly understood.

Oryzaephilus surinamensis, a grain pest beetle, harbors the highly specialized bacterial endosymbionts *Shikimatogenerans silvanidophilus*. The endosymbionts supplement the host with tyrosine and L-DOPA precursors, crucial metabolites further metabolized for cuticle melanisation and sclerotization. However, when subjected nutritionally unbalanced diets, or pharmacological inhibition of the synthesis of tyrosine or L-DOPA molecules, the symbiont titer was observed to change. This suggests a regulatory mechanism wherein metabolite intermediates in the biosynthesis pathway of the cuticle may exert influence over the regulation of host-symbiont interactions.

In this project, the molecular mechanism behind the endosymbiont regulation is investigated using transcriptomic analysis of RNA sequencing generated data, differential gene expression analysis and dietary manipulation assays. We try to identify receptors or effector genes involved in the regulation mechanism from the RNA-seq data. Additionally, we subject the beetles to a diet containing pharmacological modulators of key enzymatic processes and study the changes in the symbiont titer and the cuticle phenotype to resolve the regulatory mechanisms in the endosymbiont maintenance.

Arabidopsis thaliana vtc2-1 mutant reduces aphid survival via metabolic shifts

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Vitamin C serves multiple roles in plants, functioning as an antioxidant and a modulator of stress response. The vitamin C deficient *vtc2-1* mutant of *Arabidopsis thaliana* has been shown to exhibit resistance to the generalist aphid *Myzus persicae*. Here, we investigated its impact on the specialist aphid *Brevicoryne brassicae* and explored potential underlying mechanisms on how reduced vitamin C levels may affect aphid performance. Our findings demonstrated that *vtc2-1* plants similarly impaired the survival of *B. brassicae*. Untargeted metabolic profiling revealed significant changes in both primary and secondary metabolites in the *vtc2-1* mutant compared to the wild type, Col-0. These metabolic differences were further enhanced by aphid infestation. Specifically, *vtc2-1* exhibited elevated sugar levels. This can be linked to upregulation of upstream genes in the vitamin C biosynthesis pathway, which overlap with processes involved in polysaccharide synthesis. In addition, an increase in phenylpropanoids, particularly sinapic acid was associated to reduced aphid survival. We propose that the enhanced sugar flux in *vtc2-1* has a potential to induce osmoregulatory stress in aphids, exacerbating the effects of heightened defense metabolites. Together, the herewith described metabolic alterations could provide a mechanistic basis for the increased aphid resistance observed in the *vtc2-1* mutant.

SaxA-mediated degradation of antimicrobial 4MSOB-ITC functions as a public good for a leaf bacterial community

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Healthy leaves host both commensal and opportunistic pathogenic bacteria whose interactions help mediate balance. To eventually control this balance by biocontrol agents, it is important to understand the drivers of interactions. Isothiocyanates (ITCs) are glucosinolate degradation products in Arabidopsis thaliana that are toxic to most bacteria, but which can be detoxified by the ITC hydrolase SaxA. We hypothesized that the SaxA-mediated degradation of 4-methylsulfinylbutyl ITC (4MSOB-ITC) by the opportunistic pathogen Pseudomonas sp. 3D9 (Ps) functions as a public good by protecting cocolonizing commensals. This is important, because commensal growth would compete with Ps for resources, preventing it from causing disease. Five bacterial commensals isolated along with Ps from healthy A. thaliana leaves were characterized for their response to ITCs and growth with or without Ps or a Ps $\Delta saxA$ mutant. We use a generalized consumer-resource model incorporating ITC toxicity. substrate utilization and ITC degradation rate to predict coculture growth. A rescue index based on the models illustrates under which conditions SaxA functions as a public good. By varying degrader:commensal ratio, ITC degradation and ITC susceptibility in silico we aim to define an optimal commensal community which benefits from SaxA-mediated ITC degradation while keeping the pathogen on low levels to not harm the plant. This may help to understand how balance in the leaf microbiome arises.

Body temperature differences between green and brown grasshoppers do not result from thermal physiology or thermal preferences

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Colour polymorphisms correspond to the co-occurrence of several distinct colour morphs that vary in hue and/or brightness, independently of sex, age or any other state-dependent modifiers. Colour morphs could represent different life-history strategies, maximising their fitness locally in the trait space through trade-offs between all their biological functions. This mechanism could play a role in the maintenance of the green-brown polymorphism in Orthoptera. Grasshoppers are characterized by a widespread greenbrown polymorphism and continuous variability in brightness within colour morphs. It has previously been found that brown individuals are warmer in the field than green conspecifics, but it is unclear if these differences are related to thermal physiology and/or thermal preferences. Therefore, we experimentally tested the thermal physiology and thermal preferences of three green-brown polymorphic species of acridid grasshoppers. We found no differences between green and brown grasshoppers, either in heat-up and equilibrium temperature patterns, or in temperature preferences. Nor did we find support that the brightness variation is involved in the thermal physiology of these species. Instead, we show that body mass shapes the thermal physiology, with heavier individuals heating more slowly, and that males heated up faster and reached higher equilibrium temperatures than females. As females are heavier than males, the sex differences might be largely explained by size differences. Our results suggest that neither the thermal physiology nor the thermal preferences explain temperature differences in the field. However, green and brown individuals might still select different microhabitats in the field, which may indirectly lead to differences in body temperature. The persistence of the green-brown polymorphism may result from other mechanisms such as niche partitioning via microhabitat choice, mating preferences or frequency-dependent apostatic selection.

How to befriend your enemy: Deciphering steps of mutualism establishment in a beetle-endosymbiont system

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Microbes are associated with a wide range of taxa and often provide specific survival advantages to the host, such as the provision of essential amino acids and vitamins, detoxification, and protection against predators. At the same time, these associations benefit the microbes by ensuring a steady supply of nutrients and a stable environment. Since the preestablished host-endosymbiont relationships are intricately woven, the host often does not survive without its endosymbiont, making it difficult to retrace the initial steps towards mutualism. Therefore, while the characteristics of host-endosymbiont interactions are well-documented, the mechanisms facilitating the transition from a free-living microbe to a mutualistic endosymbiont remain elusive. The saw-toothed grain beetle Oryzaephilus surinamensis harbouring the endosymbiont Candidatus Shikimatogenerans silvanidophilus in its bacteriomes is a promising study model to investigate this missing link. The endosymbiont has a highly reduced genome but retains the Shikimate pathway, crucial for supplying precursors for tyrosine biosynthesis. Tyrosine, being essential for cuticle melanisation and sclerotisation, is critical for the beetle to enhance desiccation resistance in a dry environment, making the host heavily reliant on the endosymbiont. Sodalis praecaptivus (Sodalis) is a close relative and putative environmental progenitor of the widely distributed insect-associated Sodalis-allied symbionts and serves as an ideal candidate for experimentally probing this transition. Sodalis, a genetically tractable microbe with partial pathogenicity to O. surinamensis, can spread systemically, colonise the host bacteriome by replacing the preexisting endosymbiont, and be vertically transmitted. Since slower-growing bacteria are more likely to persist within the host without inducing excessive damage, they have the potential to facilitate long-term symbiotic integration. The project aims to establish a less pathogenic, benign Sodalis interaction in O.surinamensis by genetically manipulating key metabolic and regulatory pathways that influence its virulence, growth rate, and overall fitness within the host.

Dimerization of bisindole alkaloids in Madagascar periwinkle *C. roseus*

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Catharanthus roseus (Madagascar periwinkle) produces a variety of terpenoid indole alkaloids, many of which possess biological activities of pharmacological importance. The anticancer drugs vinblastine and vincristine are bisindole alkaloids that are formed through the dimerization of the two monoterpenoid indole alkaloid (MIA) moieties vindoline and catharanthine. In *C. roseus*, the dimers are present at a low abundance and due to their complex chemical structure, total synthesis is difficult. Thus, production of the drugs relies on isolation of the monomeric precursors from plants and subsequent coupling in vitro. The biosynthetic pathway of vindoline and catharanthine in *C. roseus* has been elucidated. However, the mechanism of dimerization and conversion to vinblastine or vincristine remains unclear.

Previous research has shown that irradiation of *C. roseus* with near ultraviolet (NUV) light can lead to increased amounts of bisindole alkaloids. Furthermore, in vitro reactions of vindoline, catharanthine, and flavin mononucleotide in presence of NUV light can cause the formation of a coupling iminium. Incubation with various peroxidases can yield the same product.

An increase in bisindole alkaloids in response to NUV exposure occurs specifically in the apoplastic fluid, as has been demonstrated by internal results. Moreover, a change in apoplastic protein composition was observed. Accordingly, our current focus is proteome analysis of the apoplastic fluid, looking to identify candidate genes for the coupling of vindoline and catharanthine, as well as the subsequent reactions forming vinblastine and vincristine. The model plant *Nicotiana benthamiana* serves as a platform for protein expression and purification for in vitro screening, as well as additional in planta characterization of candidates. Ultimately, elucidating the final steps of the biosynthetic production of vinblastine and vincristine could increase accessibility to these anticancer drugs.

Roles of volatile organic compounds in tri-trophic interaction along elevational gradient of *Salix* species

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Volatile organic compounds (VOCs) are known to play important roles in mediating tri-trophic (plantherbivore-predator) interactions. Such interactions alter under the influence of biotic and abiotic factors. Elevational gradients in nature present a special natural environment where biotic conditions are stronger at lower elevations and abiotic conditions at higher elevations. Additionally, these environments also provide hybrid zones where different plant species can crossbreed and create hybrids. Hybridization as well brings variation in VOC emission patterns and consequently in tri-trophic interactions of hybrids. However, the knowledge gap on the role of VOCs in mediating tri-trophic interactions on different species and their hybrids along an elevational gradient persists. In this study, we examined the response of two alpine hybrid willow systems to phytohormone (methyl jasmonate) induction, focusing on changes in their VOC emissions. We tested whether these changes influenced the predation patterns on artificial caterpillars of different colours (green, brown, yellow with brown) along an elevational gradient. We considered two hybrid systems in our study: 1) Salix appendiculata and S. caprea as parental species, with S. appendiculata x caprea as their hybrid; 2) S. mielichhoferi and S. myrsinifolia with S. mielichhoferi x myrsinifolia hybrid. For all species and their hybrids, we observed a decline in VOC emissions with increasing elevation. At species level, changes in VOC emission patterns differed between the two systems: the hybrid S. appendiculata x caprea was found to emit higher sesquiterpenes levels compared to their parental species. In contrast, the hybrid S. mielichhoferi x myrsinifolia showed VOC profiles similar to S. myrsinifolia, one of its parent species. Predation success generally decreased with elevation, however, this trend varied by plant species and caterpillar colour in first and second hybrid system, respectively. In the first hybrid system, bird predation success was primarily explained by Salix species identity, while in the second system, caterpillar colour had a greater impact on invertebrate predation. In general, our findings revealed a positive correlation between VOC richness and, particularly, invertebrate predation. Additionally, VOC richness had a positive impact on invertebrate predation of green caterpillars, independent of elevation. Our findings suggest that the diversity of VOCs in alpine willows shapes plant defensive strategies and tri-trophic interactions, contributing to ecological flexibility along environmental gradients.

Molecular regulation and ecological consequences of chemodiversity in Solanum dulcamara

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Chemodiversity is defined as the complexity of secondary metabolites that plants can produce and their variation in composition across spatial and temporal scales, in response to biotic and abiotic conditions (Wetzel & Whitehead, 2020). Chemodiversity is essential in plant interactions with pathogens, enemies, and mutualistic relationships, being a key player in the interaction networks. However, the evolutionary causes of such diversification and the ecological impact of the complex mixture of phytochemicals produced in a single plant at a specific moment are not yet fully understood (Schneider et al., 2021).

To elucidate the ecological role of chemodiversity, we study the diversification of steroidal glycosides (SGs) in *Solanum dulcamara*, a wild plant that grows in different environments. SGs are constitutively expressed compounds that play a crucial role in the plant's defense strategy and survival (Calf et al., 2018). It has been documented that the steroidal glycosides diversity is associated with plant ontogeny and organ type (Anaia et al., 2024).

In this project we aim to 1) evaluate organ-specific (root, leaves, and flowers) and life-stage (vegetative and flowering stage) chemodiversity composition across 45 *S. dulcamara* accessions from different geographic regions; 2) elucidate the relationship between *S. dulcamara* root chemodiversity, its belowground interactions and induced resistance to herbivores in the aboveground level; and 3) understand the molecular regulation of SGs that occurs at the intra-plant level.

We are employing LC-MS/MS-based untargeted metabolomics coupled to multivariate data analysis to access the chemical profile associated with 45 accessions of *S. dulcamara* kept in our in-house collection. Preliminary results evidence that the accession TW12 showed a higher concentration of solasodine and α -solasonine in all its organs, compared to ZD11 and ZD04.

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Regulation of the supply of the universal precursor molecules of terpenoid biosynthesis, isopentenyl diphosphate and dimethylallyl diphosphate, by the MEP and MVA pathway in two woody plant species

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Terpenoids are the most structurally diverse group of plant metabolites. Dimethylallyl diphosphate (DMADP) and isopentenyl diphosphate (IDP) serve as the universal C5-precursors in terpenoid biosynthesis, synthesized via the plastidial methylerythritol 4-phosphate (MEP) and cytosolic mevalonate (MVA) pathways. To investigate the regulation of terpenoid biosynthesis at this stage of terpenoid biosynthesis in woody plants, two species were used, the gray poplar (*Populus × canescens*) and Norway spruce (*Picea abies*).

For that, leaves at different developmental stages were investigated. The results showed that in poplar the content of MEcDP, an intermediate of the MEP pathway, was highest in young leaves, whereas the expression levels of the MEP pathway–related genes *PcDXS2* (1-deoxy-D-xylulose 5-phosphate synthase), *PcMDS1* (2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase), and *PcHDR2* (4-hydroxy-3-methylbut-2-enyl diphosphate reductase) were higher in older leaves. This discrepancy could be due to young leaves prioritizing growth or chlorophyll biosynthesis while older leaves focus on defense, but this requires further investigation. In contrast, the MVA pathway–related gene *PcHMGR* (3-hydroxy-3-methylglutaryl-CoA reductase) exhibited its highest expression in young leaves. Future experiments will quantify MVA intermediates to clarify how both pathways contribute to the terpenoid biosynthesis during leaf development. Preliminary experiments on spruce indicate that young needles also have the highest MEcDP levels, which needs to be confirmed by further studies on intermediate metabolite levels and gene expression in both pathways.

In addition to its role as an MEP intermediate, MEcDP also functions as a retrograde signal from the plastid to the nucleus that can influence plant hormone pathways and thus plant defense responses. Since MEcDP levels are higher in transgenic poplar plants in which the expression of the gene *PcHDR2*, which encodes the last step of the MEP pathway, has been silenced, these plants could serve as an ideal tool to investigate this issue. We found that the levels of jasmonic acid (JA), Jasmonoyl-L-isoleucine (JA-IIe), 12-Hydroxyjasmonic Acid (OH-JA), and 12-Hydroxyjasmonoyl-L-isoleucine (OH-JA-IIe) did not differ between the *PcHDR2*-silenced poplar lines and control plants. However, salicylic acid (SA) content was surprisingly lower in the *PcHDR2*-silenced lines compared to control plants. These results indicate that while the levels of jasmonic acid and its derivatives remain unchanged in *PcHDR2*-silenced poplar lines, the significant reduction in SA content suggests that MEcDP accumulation may selectively regulate SA-mediated defense signaling.

The formation of iridoid trail pheromones in ants

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Iridoids are cyclopentane[c]-pyran monoterpenoids that are widely distributed in plants and insects. They are mainly considered as defense compounds, but also act as pheromones in insects. While the biosynthesis of iridoids in plants has been intensively studied, little is known about how insects produce these compounds. In this project, we aim to study the formation of iridoids, namely iridomyrmecin and dolichodial, in two different ant species, the Argentine ant (Linepithema humile), native to South America but now invasive in many Mediterranean climates, and the Australian Meat ant (Iridomyrmex purpureus). By comparing the transcriptome of the abdomen, the site of iridoid production, with that of the head and thorax of adult workers, we identified genes potentially involved in iridoid biosynthesis in both species. Furthermore, by isolating the pygidial gland of *I. purpureus*, we were able to generate a transcriptome of this iridoid-producing tissue. We are now characterizing the candidate genes by heterologous expression in different host systems such as *Escherichia coli*, yeast, and insect cell lines, followed by in vitro enzyme assays. We could already show that one of the candidate genes, which is highly expressed in the abdomen of L. humile workers as well as in the pygidial gland of I. purpureus, encodes a functional isopentenyl diphosphate synthase capable of producing the iridoid precursor geranyl diphosphate (GPP). We have also identified a phosphatase that catalyzes the formation of geraniol from GPP, a P450 capable of converting geraniol into 8-hydroxygeraniol, and an oxidoreductase that oxidizes 8hydroxygeraniol to 8-oxogeranial in in vitro assays. We are currently investigating potential iridoid synthase candidates and a number of oxidoreductases that may play a role in later steps of iridoid biosynthesis.

The role of recurrent long- and short-range connections in experiencedependent modulation in *Drosophila*

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Odor-driven innate behaviors dictate an animal's survival and reproduction. Life decisions in Drosophila melanogaster, such as mating, oviposition choice and parasitoid avoidance are also triggered by specific olfactory cues that activate hard-wired neuronal circuits leading to stereotyped behaviors. The hedonic valence and intensity of such odors are known to be encoded in the lateral horn of the fly brain. However, depending on new experiences, physiological state and context, the representation of stimuli can be overwritten to assign new values, allowing the fly to adapt to changing environments. In a novel ecologically relevant oviposition assay, we show that mated female flies innately prefer parsnip food for oviposition, but change their preference to apple food after oviposition training, e.g. after having oviposited on the apple food (Otarola-Jimenez et al., 2024). Also, when these flies are tested in a Ymaze assay, apple-trained flies choose the apple odor over the parsnip odor. Results from these behavioral experiments demonstrate that even innate olfactory behaviors are modulated by prior experience. We propose that this modulation occurs in primary and secondary brain centers mediated by recurrent connections between these olfactory neuropils (i.e. antennal lobe - lateral horn - mushroom body). Using the recently established oviposition learning as a behaviorally relevant learning paradigm, we are currently monitoring the modulation of second- and higher-order neurons via two-photon functional imaging. Furthermore, based on the published Drosophila brain connectome data, we are analyzing the role of candidate centrifugal and modulatory neurons for mediating this modulation through recurrent connections within and between olfactory neuropils.

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