



# 23<sup>rd</sup> IMPRS Symposium

April 17-18, 2024

Altes Schloss Dornburg, Max-Krehan-Straße 4, 07774 Dornburg

Please join us for:

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

## Guest speakers



**Laura Flórez**

Department of Plant  
and Environmental  
Sciences  
University of  
Copenhagen,  
Denmark



**Meredith Schuman**

Department of  
Geography  
University of Zurich,  
Switzerland

# Program

Wednesday, 17<sup>th</sup>, 2024

	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	Welcome (Großer Kaisersaal) <i>Claudia Voelckel</i>
09:30	New arrivals and defended theses in 2023/2024 (Großer Kaisersaal) <i>Sarah O'Connor</i>
09:45	Plenary Lecture 1 (Großer Kaisersaal) "Exploring defensive symbiosis in insects - as an early career scientist" <i>Laura Flórez, University of Copenhagen, Denmark</i> <a href="#">Abstract</a> page 9 <span style="float: right;"><i>Chair: Martin Kaltenpoth</i></span>
10:30	<b>Coffee break</b> (Rittersaal)

<b>1<sup>st</sup> talk session</b> (Großer Kaisersaal) <i>Chair: Sthandiwe Nomthandazo Kanyile</i>	
11:00	1 - Influence of the facultative bacterial endosymbiont <i>Regiella insecticola</i> on the performance of the pea aphid <i>Acyrtosiphon pisum</i> <i>Kristina Schädel (Department of Biochemistry, MPI-CE)</i> <a href="#">Abstract</a> page 11
11:15	2 - Unveiling the scent evolution in plants and seed dispersers <i>Linh Nguyen (German Centre for Integrative Biodiversity Research - iDiv)</i> <a href="#">Abstract</a> page 12
11:30	3 - Don't put all eggs into one basket! Oviposition dynamics in a hawkmoth <i>Eduardo Briceño Aguilar (Department of Evolutionary Neuroethology, MPI-CE)</i> <a href="#">Abstract</a> page 13
11:45	4 - Exploring seasonal, vertical, and sexual dimensions in the chemodiversity of mature <i>Populus nigra</i> trees <i>Sol Yepes Vivas (Department of Biochemistry, MPI-CE)</i> <a href="#">Abstract</a> page 14
12:00	5 - Seasonal changes in <i>Drosophila melanogaster</i> microbial communities and its implications on the host cold tolerance <i>Marion Lemoine (Department of Insect Symbiosis, MPI-CE)</i> <a href="#">Abstract</a> page 15
12:15	<b>Lunch break</b> (Rittersaal)

<b>1<sup>st</sup> poster session (odd numbers)</b>	
<i>Chair: Claudia Voelckel</i>	
13:30	One minute poster talks (1,3,5,7,9,11,13,15,17,19,21) (Großer Kaisersaal)
13:45 – 15:15	Poster session (Kleiner Kaisersaal & Flur)
15:15	<b>Coffee break</b> (Rittersaal)
15:15 – 15:45	IMPRS faculty meeting (Bibliothek 1)

<b>2<sup>nd</sup> talk session</b> (Großer Kaisersaal)	
<i>Chair: Julio Antonio Otarola Jimenez</i>	
15:50	6 - Environmental and genetic drivers in an insect wing dimorphism <i>Lilian Cabon (Population Ecology Group, Institute of Ecology and Evolution, FSU)</i> <a href="#">Abstract</a> page 16
16.05	7 - Evolution of the olfactory system in feeding versus non-feeding moths: from receptors to glomeruli <i>Marina F. Quadrado (Department of Evolutionary Neuroethology, MPI-CE)</i> <a href="#">Abstract</a> page 17
16:20	8 - Investigating metabolic interactions in a tripartite symbiosis <i>Camila Pereira (Department of Insect Symbiosis, MPI-CE)</i> <a href="#">Abstract</a> page 18
16:35	9 - Spreading dynamics in clonal raider ant interaction networks <i>Sarah Rogoz (Lise Meitner Group Social Behavior, MPI-CE)</i> <a href="#">Abstract</a> page 19
16:50	<b>End</b>
	<b>Travel by regional train from Dornburg/Saale to Jena Paradies</b> <b>RB 25 to Halle/Saale leaves at 17:31 in Dornburg/Saale (Jena-Zwätzen 17:37, Jena Saalbahn) and arrives in Jena Paradies at 17:43</b>

## Thursday, April 18<sup>th</sup>, 2024

	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 Saalbahn) 8:17, Jena-Zwätzen 8:20) and arrives in Dornburg at 8:27 a.m.
09:15	Plenary Lecture 2 (Großer Kaisersaal) “Spatial genetics of plant communities: Locating the living potential of plants” <i>Prof. Dr. Meredith Schuman, University of Zurich, Switzerland</i> <a href="#">Abstract</a> page 10
	<i>Chair: Rayko Halitschke</i>

10:00	<b>Coffee break</b> (Rittersaal)
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### 3<sup>rd</sup> talk session

Chair: Iulia Barutia

10:30	10 - Beyond defense: Glucosinolate structural diversity shapes recruitment of a metabolic network of leaf-associated bacteria <i>Kerstin Unger (Plant Microbiosis Group, Institute for Microbiology, FSU)</i> <a href="#">Abstract</a> page 20
10:45	11 - Creating a genetically tractable symbiosis: Experimental symbiont replacement in grain pest beetles <i>Ronja Krüsemer (Department of Insect Symbiosis, MPI-CE)</i> <a href="#">Abstract</a> page 21
11:00	12 - Azelaic acid: A distress signal in plant-microbe Interaction <i>Priya Reddy (Department of Plant Physiology, FSU)</i> <a href="#">Abstract</a> page 22
11:15	13 - The formation of iridoid trail pheromones in ants <i>Maithili Datta (Department of Natural Product Biosynthesis, MPI-CE)</i> <a href="#">Abstract</a> page 23
11:30	<b>Lunch break</b> (Rittersaal)

### 2<sup>nd</sup> poster session (even numbers)

Chair: Claudia Voelckel

12:30	One minute poster talks (2,4,6,8,10,12,14,16,18,20) (Großer Kaisersaal)
12:45 – 14:15	Poster session (Kleiner Kaisersaal & Flur)
14:15	<b>Coffee break</b> (Rittersaal)

### 4<sup>th</sup> talk session (Großer Kaisersaal)

Chair: Kalpana Jain

14:45	14 - The dance of <i>Arabidopsis</i> with time, terrain, and tiny allies: Deciphering the microbiome-climate-plant triad <i>Syed Ali Komail Raza (Plant Microbiosis Group, FSU)</i> <a href="#">Abstract</a> page 24
15:00	15 - Olfactory signal guided behavior in <i>Locusta migratoria</i> <i>Anjana Unni (Department of Evolutionary Neuroethology, MPI-CE)</i> <a href="#">Abstract</a> page 25
15:15	16 - External fungal symbionts increase performance of the Eurasian spruce bark beetle <i>Emily Puckett (Department of Biochemistry, MPI-CE)</i> <a href="#">Abstract</a> page 26
15:30	17 - Plant diversity and community history shapes <i>Plantago lanceolata</i> metabolome <i>Pamela Medina van Berkum (Department of Biochemistry, MPI-CE)</i>

	<a href="#">Abstract</a> page 27
15:45	<b>End</b> 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 16:31 or 17:31 in Dornburg/Saale (Jena-Zwätzen 16:37/17:37, Jena Saalbahnhof) and arrives in Jena Paradies at 16:43/17:43.

<b>PhD meeting</b> (MPI for Chemical Ecology)	
18:00	<b>Dinner (Cafeteria)</b>
18:30	Introduction PhD representatives (A1.011 + A1.009)
19:00	Award ceremony <i>Sarah O'Connor</i>

# Posters

1. Gene clustering in barley reveals cryptic oxidative rearrangement in gramine biosynthesis  
Sara Leite Dias (*IPK Gatersleben*), [Abstract](#) page 29
2. Symbiont-symbiont interactions in a defensive symbiosis  
Alina Nick (*Department of Insect Symbiosis, MPI-CE*), [Abstract](#) page 30
3. Mechanism of metabolic regulation in grain pest beetle-endosymbiont interaction  
Soumi Bhattacharyya (*Department of Insect Symbiosis, MPI-CE*), [Abstract](#) page 31
4. Immune division of labour in the clonal raider ant  
Zimai Li (*Lise Meitner Group Social Behavior, MPI-CE*), [Abstract](#) page 32
5. Tissue-specific changes in young *Populus nigra* trees chemodiversity under *Lymantria dispar* herbivory  
Sol Yepes Vivas (*Department of Biochemistry, MPI-CE*), [Abstract](#) page 33
6. Imaging unimaginable symbiotic dynamics in a grain pest beetle  
Dongik Chang (*Department of Insect Symbiosis, MPI-CE*), [Abstract](#) page 34
7. Evolutionary history of tyrosine-supplementing endosymbionts in pollen-feeding Dasytinae (Coleoptera: Melyridae) beetles  
Jürgen C. Wierz (*Department of Insect Symbiosis, MPI-CE*), [Abstract](#) page 35
8. Life with long appendages: Functional, kinetic and morphological adaptations in house centipedes  
Iulia Barutia (*Institute for Zoology and Evolutionary Research, FSU / Research Group Olfactory Coding, MPI-CE*) [Abstract](#) page 36
9. Genetic determination of color polymorphisms in grasshoppers  
Xinyi Cheng (*Population Ecology Group*) [Abstract](#) page 37
10. Nematode infections reshape ant chemical signatures  
Bhoomika Bhat (*Lise Meitner Group Social Behavior, MPI-CE*), [Abstract](#) page 38
11. Elucidating the biosynthetic pathway of *Cinchona* alkaloids: Identification of a dihydrocorynantheol synthase  
Tingan Zhou (*Department of Natural Product Biosynthesis, MPI-CE*), [Abstract](#) page 39
12. Understanding alkaloid biosynthesis in ladybird beetles  
Angeliki Stathaki (*Department of Natural Product Biosynthesis, MPI-CE*), [Abstract](#) page 40
13. The role of *R. insecticola* in pea aphid performance and plant defense response  
Surhut Sant (*Department of Biochemistry, MPI-CE*), [Abstract](#) page 41
14. Untargeted metabolomics with electron ionization: A roadmap for adapting SIRIUS and beyond to GC-EI mass spectrometry data  
Jonas Emmert (*Bioinformatics, Inst. for Computer Science, FSU*), [Abstract](#) page 42
15. Molecular basis and specificity of symbiont colonization in *Lagria villosa*  
Johann de Beer (*Department of Insect Symbiosis, MPI-CE*), [Abstract](#) page 43
16. Are you sick? - The chemical and sensory bases of social immunity in the clonal raider ant  
Sandra Tretter (*Lise Meitner Group Social Behavior, MPI-CE*), [Abstract](#) page 44
17. Mating experience changes the oviposition preference of *Drosophila melanogaster*  
Julio Otárola-Jiménez (*Department of Evolutionary Neuroethology, MPI-CE*), [Abstract](#) page 45
18. Individual and social immunity in the clonal raider ant  
Xiaohua Chu (*Lise Meitner Group Social Behavior, MPI-CE*), [Abstract](#) page 46
19. Investigating the costs and benefits of a host plant-dependent chemical defense in a herbivorous insect  
Johannes Körnig (*Department of Insect Symbiosis, MPI-CE*), [Abstract](#) page 47

20. Host control in symbiont cell morphology: decoding the intricacies of beewolf wasp's symbiont transmission  
*Bernal Matarrita-Carranza (Department of Insect Symbiosis, MPI-CE), [Abstract](#) page 48*
21. Arabidopsis foliar bacteriome under simulated winter  
*Syed Ali Komail Raza (Plant Microbiosis, FSU), [Abstract](#) page 49*

# Talks





# Plenary Lecture 1

## Exploring defensive symbiosis in insects — as an early career scientist

Laura V. Flórez<sup>1,2</sup>, Rebekka Janke,<sup>2,3</sup> Ramya Ganesan<sup>2,4</sup>, Laura Lender<sup>5</sup>, Filip Kaftan<sup>6</sup>, Jürgen Wierz<sup>2,4</sup>, Sam Waterworth<sup>6</sup>, Kirstin Scherlach<sup>7</sup>, Aleš Svatoš<sup>5</sup>, Jason Kwan<sup>6</sup>, Christian Hertweck<sup>7</sup>, André Rodrigues<sup>8</sup>, Henrik de Fine Licht<sup>1</sup>, Martin Kaltenpoth<sup>2,4</sup>

<sup>1</sup>Department of Plant and Environmental Sciences, University of Copenhagen

<sup>2</sup>Department of Evolutionary Ecology, Johannes Gutenberg University, Mainz

<sup>3</sup>Department of Symbiosis, Max Planck Institute for Marine Microbiology

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<sup>7</sup>School of Pharmacy, University of Wisconsin-Madison, USA

<sup>8</sup>Department of Biomolecular Chemistry, Leibniz Institute for Natural Products Research and Infection Biology, HKI

<sup>9</sup>Department of Biochemistry and Microbiology, UNESP-São Paulo State University, Rio Claro, Brazil

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Pathogenic fungi stand out as predominant natural enemies of insects. While insects are generally equipped to hinder fungal infections, many resort to microbial symbionts that enhance protection or provide novel strategies to effectively counteract antagonistic fungi. However, relying on defensive symbionts presents its own set of challenges. Establishing a stable association involves intricate mechanisms for selecting, harboring, regulating, and transmitting these microbial partners. How defensive symbioses evolve and how the interplay with fungal antagonists might shape this process are focal points in my research. To address these questions, I have delved into two insect hosts employing distinct strategies to acquire and accommodate bacterial partners. In *Lagria* beetles, we've learned about mechanisms to maintain a consistent community of ectosymbionts. This includes the presence of antibiotic-producing bacteria, crucial for safeguarding vulnerable life stages of the insect against the entry of potential threats. Contrastingly, in the case of western flower thrips, resident bacteria within the gut could reinforce resistance against fungal pathogens through indirect mechanisms. Also, the environmental context and the plant hosting the thrips are likely pivotal factors determining the microbial interactions that affect the insect.

During the talk, I will tell you about the scientific journey through which we have explored these complex insect-microbe interactions. My experience navigating this journey began during my time as a doctoral student at the IMPRS, so I will also share a personal perspective on the challenges and opportunities encountered along the way.

## Plenary Lecture 2

### Spatial genetics of plant communities: Locating the living potential of plants

Meredith C. Schuman<sup>1,2</sup> and the Spatial Genetics team

<sup>1</sup>Department of Geography and <sup>2</sup>Department of Chemistry, University of Zurich, Zurich, Switzerland  
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As conditions for life on Earth change at an increasingly rapid pace, the ability to adapt to these changes becomes ever more important for the persistence of species and the functioning of their communities. Genetic diversity increases a species' chances to adapt to changing conditions, if that variation is available in the right place and at the right time, i.e., distributed both within and across populations over a relatively large geographic range. Distributions of standing genetic variation are especially important for the adaptation of species having generation times that are long in comparison to the pace of global change, such as trees and many other plants that form the trophic and habitat "base" for most terrestrial and many aquatic ecosystems. Yet it is not sufficient simply for genetic variation to be widespread – enough variants must encode adaptive traits. If we want to predict how species and their populations will fare under global change, we must map spatial genetic variation and study the complex processes linking it to both organismal and ecosystem functioning in changing environments. Such studies are needed to inform conservation and management strategies and are increasingly mandated by policy. There is, however, a pernicious hurdle to cross: even with rapid developments in genetic sequencing and other "omics" technologies, these measurements do not scale. The expense of sampling and analysing sequence data increases approximately linearly with an increasing number and spread of sampled populations, and upscaling also carries a steep price for trait measurements requiring tissue sampling and sample preparation and analysis. Fortunately, remote sensing offers a toolkit well equipped to scale the information gleaned from precious *in situ* samples, and to map this information onto spatial distributions and environmental gradients. Combining these approaches allows us to study changing distributions of environmental, functional, and even genetic variation in space and time. The Spatial Genetics group uses this interdisciplinary toolkit to study plants in several systems important to human well-being: subsistence agriculture, viticulture, managed forests, polluted lakes, and wild landscapes. In this talk, I will present the story of how my path as a chemical ecologist took me from molecular genetics to geography, and share some discoveries from the first five years of Spatial Genetics.

# Talk 1

## Influence of the facultative bacterial endosymbiont *Regiella insecticola* on the performance of the pea aphid *Acyrtosiphon pisum*

Kristina Schädel<sup>1</sup>, Benjamin Weiss<sup>2</sup>, Veit Grabe<sup>3</sup>, Linus Wegner<sup>4</sup>, Alexandra C.U. Furch<sup>4</sup>, Martin Kaltenpoth<sup>2</sup>, Jonathan Gershenzon<sup>1</sup> and Grit Kunert<sup>1</sup>

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The pea aphid (*Acyrtosiphon pisum*) is a species complex consisting of at least 15 distinct host races, each specialized on one or a few plants of the legume family. However, all host races can feed and survive on the universal host plant *Vicia faba*. One of the main questions is how the different host races evolved and what enables them to feed on their respective host plants.

As many herbivorous insects, the pea aphid harbors endosymbiotic bacteria. One is the obligate endosymbiont, *Buchnera aphidicola*. It lives in bacteriocytes near the gut and provides essential amino acids to the aphid. In addition, many pea aphid host races also harbor other bacteria as facultative endosymbionts, such as strains of *Regiella*, *Serratia*, and *Rickettsia*. The pea aphid host race specialized on clover is mainly associated with *Regiella insecticola*, an endosymbiont that lives extracellularly in the hemolymph of the pea aphid.

We wanted to know whether the presence of *R. insecticola* influences the performance of aphid clones belonging to the *Trifolium* race. Therefore, we investigated the performance of two different aphid clones belonging to the *Trifolium* race. From these aphid clones, we tested aphids naturally carrying *R. insecticola*, the corresponding aphids without *R. insecticola*, and former *Regiella*-free aphids that I reinjected with *R. insecticola*. All aphids were tested on their preferred host plant, *T. pratense* and the universal host plant, *V. faba*. We monitored several life history traits and we localized *R. insecticola* and *B. aphidicola* by FISH to verify that our reinjected pea aphid lines were appropriate positive controls.

The influence of *R. insecticola* differed depending on the investigated performance trait, the plant on which the aphid lived, and on the aphid clone. For one aphid clone, we found a strong negative influence of *R. insecticola* on several life history traits, but mainly when the clone lived on *V. faba*. Regardless of the presence of *R. insecticola*, aphids feeding on the *T. pratense*, the host plant to which they are adapted on, had a higher survival rate than aphids feeding on the universal host plant.

Our endosymbiont localization study reveal that pea aphids reinjected with *R. insecticola* were appropriate positive controls, but surprisingly we also detected the obligate endosymbiont *B. aphidicola* unexpectedly in the hemolymph and in the mouth part of the aphids. It cannot be excluded that the endosymbionts might enter the plant and influence the plant's defense response.

## Talk 2

### Unveiling the scent evolution in plants and seed dispersers

Linh M. N. Nguyen<sup>1,2,3</sup>, Diary Razafimandimby<sup>4</sup>, Rebekka Sontowski<sup>5</sup>, Jana Ebersbach<sup>6</sup>, Renske Onstein<sup>1,7</sup>, Philipp Schluter<sup>8</sup>, Nicole van Dam<sup>1,2,3,5</sup>, Omer Nevo<sup>1,2</sup>

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Fruits have evolved to be attractive to seed dispersers, and fruit traits evolved in response to selection pressures by frugivores. Fruit scent has evolved as a signal for frugivores, signaling ripeness and potentially fruit quality. However, it is still unclear how and what parts of fruit scent have evolved as signals for frugivores and what information they contain. Unlike most chemical classes, aliphatic esters are found to be concentrated in species that communicate with seed dispersers via chemical cues and only in ripe fruits. Some evidence and theoretical basis have indicated a positive correlation with sugar content. This means that there might be a link between chemical signals and where chemical coevolution is expected for fruit dispersed in lemurs. Our research aims to identify whether aliphatic esters are indeed an honest signal for fruit quality and test whether it is an adaptation acquired by plants. We test this hypothesis on a model system of up to 20 fig species (*Ficus* spp; MORACEAE) growing in Madagascar. With collections of fruit and leaf, we intend to (1) establish an ecological network, (2) reconstruct phylogeny, (3) identify the link between chemical signal and reward by using thermo desorption gas chromatography-mass spectrometry (TD-GCMS) and high-performance liquid chromatography (HPLC) to test whether it is the unique link between species and dispersal ecology, and (4) to sequence alcohol acyltransferase (AAT) to check if the selection regimes corresponding to the dispersal mode.

## Talk 3

### Don't put all eggs into one basket! Oviposition dynamics in a hawkmoth

Eduardo Briceño-Aguilar<sup>1</sup>, Bill S. Hansson<sup>1</sup>, Markus Knaden<sup>1</sup>

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Oviposition choice in insects can be influenced by information acquired from visual, olfactory, and gustatory cues. Most studies focusing on oviposition in lepidopterans have examined the insect's evaluation plant suitability, the resulting subsequent plant acceptance or rejection, and learning from oviposition experience. Especially studies on oviposition learning suggest that experienced moths might exhibit oviposition constancy, i.e. despite naïve moths being generalists, they finally lay most of their eggs on a host they have already experienced. However, whether and how these insects transition between different host species to lay eggs remains poorly explored. In our study, we investigated the tobacco hawkmoth, *Manduca sexta*, with two well-known hosts (Solanaceae) and a less studied one (Martyniaceae). When investigating their oviposition behavior in wind-tunnel and semi-natural tent assays, we observed that hawkmoths revisit both individual leaves in the wind tunnel and plant clusters in the tents several times before they search for another host. This revisitation behavior might be mediated by learning, i.e., an immediate increase in preference after experience. At the same time, such behavior is unexpected from an insect optimizing energy. Interestingly, we found that moths after leaving a cluster of a given host species the moths do not exhibit a higher preference for a second cluster of plants of the same species, suggesting that hawkmoths rather display opportunistic oviposition behavior than oviposition constancy. Ovipositing hawkmoths, travel long distances and, probably encounter high environmental variability. Therefore, host flexibility might be adaptive as it allows the moths to explore and find other suitable hosts for their progeny, and to avoid putting all eggs into one basket.

## Talk 4

### Exploring seasonal, vertical, and sexual dimensions in the chemodiversity of mature *Populus nigra* trees

Sol Yepes-Vivas<sup>1</sup>, Michael Reichelt<sup>1</sup>, Jonathan Gershenzon<sup>1</sup> & Sybille B. Unsicker<sup>1,2</sup>

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The canopies of mature trees, with their heights and wide diameters, create a mosaic of abiotic and biotic conditions. However, the impact of seasonality and vertical stratification on leaf chemodiversity within these canopies remains unexplored. Furthermore, the extent of sexual dimorphism in chemical traits among dioecious tree species is not well understood. This study delves into the spatiotemporal trajectories of leaf chemistry in adult male and female black poplar (*Populus nigra*) trees in a natural floodplain forest in northeastern Germany. Using targeted and untargeted LC-MS/MS analyses on leaves from eight trees at five canopy heights, our findings reveal the profound influence of sex, position within the canopy, and season on the chemical composition and diversity of black poplar leaves.

## Talk 5

### Seasonal changes in *Drosophila melanogaster* microbial communities and its implications on the host cold tolerance.

Marion Lemoine<sup>1</sup>, Thomas Woehner<sup>2</sup>, Martin Kaltenpoth<sup>1</sup>

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More than ten thousand years ago, *Drosophila melanogaster*, an ectotherm organism, was able to colonize new habitats in temperate regions while originating from a sub-Saharan climate. The remarkable success of the expansion the fruit fly abiotic niche is dependent on thermal tolerance mechanisms, which allow the insect to survive fluctuating temperature regimes by tolerating modifications of its metabolism and in the case of cold tolerance recovering from lethal injuries induced by a cold shock. These mechanisms are highly dependent on the insect nutritional status and survival to colder viable is known to be diet specific. In *Drosophila melanogaster*, gut microbes are acquired via food intake and have been described to participate in a multitude of metabolic functions via provisioning essential precursors and nutrients thus having an impact on the nutritional status of its host. This suggests that gut microbial communities could participate directly or indirectly in cold tolerance mechanisms in *Drosophila melanogaster*.

Here we show that fungal and bacterial communities of wild-caught fruit flies are changing through seasons and we identified specific microbial candidates for colder and warmer seasons. To test the potential implication of microbes on *Drosophila melanogaster* cold tolerance, we infected axenic flies with fungal and/or bacterial strains isolated from wild-caught flies and tested their cold tolerance. We showed that gut microbes are rescuing cold induced delay in host development rate and reduced reproductive success enabled by a combined active host-microbe interaction and a passive nutritional supplementation. We also show that gut microbes are able to rescue its host from lethal cold injuries.

Further studies aiming at understanding the mechanistic basis of such interaction are needed and necessary to predict survival of natural populations of microbe associated insects beyond our fruit fly laboratory strain, especially in the context of global change.

## Talk 6

### Environmental and genetic drivers in an insect wing dimorphism

Lilian Cabon<sup>1,2</sup>, Mahendra Varma<sup>1,2</sup>, Gabe Winter<sup>1</sup>, Anne Ebeling<sup>1</sup>, Holger Schielzeth<sup>1</sup>

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Evolutionary fitness is determined by the match between an organism's phenotype and its local environment. When mismatched, individuals may want to disperse to more suitable habitats. For flightless insects, however, the dispersal range is typically limited. Numerous short-winged species have therefore evolved wing dimorphism, that is, they can develop long wings. Wing morph may be genetically or environmentally determined, but these two drivers have rarely been analysed together. We studied the inheritance and density-dependent plasticity in the wing dimorphism of the meadow grasshopper *Pseudochorthippus parallelus*. Using a full-sib half-sib breeding design, we found that the development of long wings strongly depends on rearing density, with tactile stimulation being the most likely proximate cause. Additionally, we found significant heritable variation in the development of long wings, both in the propensity to produce long wings and in response to density. Wing dimorphism can thus evolve by natural selection. Our results have implications for the phenotype-environment match and ultimately the evolution of individualised niches. Induced dimorphisms represent a form of niche conformance and both genetic and induced dimorphisms facilitate niche choice. Our study shows that niche-related polymorphisms can evolve directly and indirectly via selection on the sensitivity threshold.



## Talk 7

### Evolution of the olfactory system in feeding versus non-feeding moths: From receptors to glomeruli

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Nocturnal animals like moths rely heavily on their sense of smell to locate food sources, with odor recognition mediated by the expression of odorant receptor (OR) genes. While most hawkmoths feed on floral nectar and are strongly attracted to the scent of night-blooming plants, closely related species have reduced mouthparts as adults and are unable to feed. We aimed to explore whether this difference has led to adaptations in the olfactory system and hypothesized that evolutionary pressures associated with locating floral nectar sources have influenced the repertoire of ORs, potentially resulting in fewer OR genes in non-feeding moths. We investigated the expression profile of ORs and the organization of the antennal lobe (AL) in feeding (n=7) and non-feeding (n=7) species. Using RNA-seq results, we annotated 64 putative OR gene transcripts and found a lower number of ORs expressed in the antennae of non-feeding than feeding species (45 vs. 52 ORs,  $p=0.009$ , Mann-Whitney U-test). Furthermore, confocal microscopy and 3D reconstructions showed that the AL of non-feeding species had fewer glomeruli and was reduced in volume compared to feeding species (67 vs. 71 glomeruli,  $p=0.001$ ; 11% vs. 15% of total brain volume,  $p=0.018$ ). This difference likely reflects the reduced olfactory abilities of non-feeding species, suggesting that they may rely on a narrower range of olfactory cues to identify host plants. These findings enhance our comprehension of moth olfactory systems and their adaptations to different life histories, particularly in taxa where such information is not yet available.

## Talk 8

### Investigating metabolic interactions in a tripartite symbiosis

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Beetles are distinguished by their hard cuticle that serves as a shield against various threats such as predators, parasites, physical injuries, and environmental stresses. A key component of cuticle synthesis and modification (sclerotization and melanization) is tyrosine, but insects are largely dependent of dietary uptake as animals never obtained the biosynthetic pathway to synthesize it on their own. Several beetle families evolved beneficial associations with symbiotic bacteria capable of producing tyrosine or at least its precursor, and thereby gained the ability to synthesize a mature fully pigmented and sclerotized cuticle under nutritionally limited conditions.

Our study focuses on investigating the symbiotic relationships within the Bostrichidae family, using *Dinoderus porcellus* as our model system due to its association with dual bacterial symbionts. The symbiont *Shikimatogenerans bostrichidophilus* retained a unique metabolic pathway: the shikimate pathway, which produces the precursor of tyrosine. The second symbiont, *Bostrichidicola ureolyticus*, retained genes involved in urea recycling and the biosynthesis of peptidoglycan, lysine, methionine, and vitamins. We test whether *Bostrichidicola* recycled nitrogen from urea is incorporated into amino acids, but specifically tyrosine synthesized by *Shikimatogenerans*, and later in the host cuticle, resulting in an exchange of metabolites between the host and endosymbionts. Furthermore, we investigate the contributions of both endosymbionts to the host physiology, by supplementing the beetle's diet with selective antibiotics, or pharmacological inhibitors for the differentially retained metabolic pathways. We evaluate the impact of the treatments on symbionts and host, by monitoring both symbionts' titers and measuring the host cuticle's thickness and melanisation.

We observed that beetles on diets supplemented with antibiotics and glyphosate exhibited a significant reduction in *Shikimatogenerans* titers, and compromised cuticle pigmentation. This finding supports our preliminary hypothesis that the host benefits from the tyrosine precursors synthesized by its symbionts. Furthermore, when the diet was supplemented with inhibitors targeting the lysine biosynthetic pathway, we observed an increase only in *Bostrichidicola ureolyticus* titers, but no impact on the cuticle. This suggests that this symbiont's contributions may extend beyond cuticle formation and could play a role in other metabolic processes within the host. By correlating dietary nutrition with symbiont titers and cuticle measurements, our study intends not only to get insights into the endosymbionts' impact on host physiology but also to uncover potential regulatory mechanisms involved in this intricate tripartite interaction.

## Talk 9

### 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 is 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.

## Talk 10

### **Beyond defense: Glucosinolate structural diversity shapes recruitment of a metabolic network of leaf-associated bacteria**

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Leaf bacteria are critical for plant health, but little is known about how plant traits control their recruitment. Aliphatic glucosinolates (GLSs) are a class of defense metabolites present in leaves of Brassicaceae plants in genotypically-defined mixtures. Upon plant cell damage, they are broken down into products that deter herbivory and inhibit pathogens. We studied the model *A. thaliana* genotype Col-0 which produces mainly 4-methylsulfinylbutyl-GLS and NG2, a genotype isolated from a local wild population, with mainly allyl-GLS. An aliphatic GLSs-free mutant is already available in Col-0 background and we generated one in NG2 background. Using bacterial growth analysis, 16S rRNA gene amplicon sequencing and metabolomics, we find that both GLSs differentially affect commensal leaf bacteria: In Col-0, GLS breakdown products are highly toxic to bacteria but have no effect on natural colonization of healthy leaves. In contrast, NG2 leaves enrich Burkholderiales bacteria, an effect also detected in nature. Indeed, intact allyl-GLS as a carbon source specifically enriched a Burkholderiales-containing community in-vitro. In this context, Burkholderiales depended on other bacteria that can metabolize GLSs. Metabolism of GLS was accompanied by detoxification of the breakdown product with different efficiency depending on the GLS chemical structure. This might help to explain how GLS structural diversity influences bacterial recruitment in diverse genotypes. Though Burkholderiales did not utilize aliphatic GLSs directly, they increased community growth rates *in vitro*. Together, these results suggest a broader role for well-studied defense metabolites in the plant microverse and may offer directions for targeted control of balance in the leaf microbiome.

# Talk 11

## Creating a genetically tractable symbiosis: Experimental symbiont replacement in grain pest beetles

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Symbiosis with microorganisms is a crucial factor in insect diversification as it can enable insect hosts to exploit new ecological niches. Unfortunately, the intimacy of many symbioses hampers research on this topic: The lack of experimental tractability of host and/or symbiont commonly prevents genetic manipulation of insect associated microbes, which is important to understand how different symbiont genotypes can affect host fitness. Sawtoothed grain beetles (*Oryzaephilus surinamensis*, Silvanidae) harbor intracellular *Shikimatogenerans silvanidophilus* bacteria that supply their insect host with tyrosine precursors, vital for the synthesis and melanization of the host's cuticle. While *S. silvanidophilus* remains unculturable, the ability to retain symbiont-free *O. surinamensis* opens opportunities for experimental manipulation: Replacing native *S. silvanidophilus* symbionts with a genetically tractable bacterium could allow for the genetic engineering of this symbiosis. *Sodalis praecaptivus* is a free-living bacterium that is closely related to several insect associated *Sodalis* species. Because it is I) culturable and amenable to genetic engineering and II) able to infect insect hosts like tsetse flies and weevils, we considered *S. praecaptivus* a suitable candidate to act as a novel symbiont in *Oryzaephilus*. To assess its infection capabilities, we injected fluorescently labeled *S. praecaptivus* into the hemolymph of reproducing females and monitored survival and infection status of the beetles and their offspring. Using live-imaging and microscopy approaches, we show that *S. praecaptivus* could establish systemic infections in the beetle host and was vertically transmitted from mother to offspring. We also present first insights into our attempts to unravel the interactions between the novel symbiont and *O. surinamensis*, focusing on how *Sodalis* influences host fitness in the parental and first offspring generation as well as investigating how introduction of *S. praecaptivus* affects native *S. silvanidophilus* symbionts. Our findings represent the foundation of establishing a genetically tractable model symbiosis that will allow for studying host-symbiont interactions in a nascent mutualism.

## Talk 12

### Azelaic Acid: A distress signal in plant- microbe Interaction

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Plant-microbial interaction is a complex web of interactions. Plants in their natural environment face drought, salinity, temperature, and other biotic stresses. When the plant perceives stress, it sends signals within the plant and equips itself to fight the stress. In this scenario, azelaic acid (AzA), a C9 distress signal molecule (“HOOC (CH<sub>2</sub>)<sub>7</sub> COOH”), plays a prominent role in plant- microbe communication. The oxylipin AzA is synthesized in plastid membranes and triggers immunity through an AZI1/EARLI1/MPK3/6-dependent pathway and systemic signal propagation within the plant; but the traverse of AzA in the plant system remains a mystery. Here in this study, I investigate the role of AzA and its receptor AZI in the signal propagation from roots to leaves. Further experiments in this direction may help unravel the grey areas of plant microbe interaction.

## Talk 13

### 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 shown 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 P450 capable of converting geraniol into 8-hydroxygeraniol and an oxidoreductase that oxidized 8-hydroxygeraniol to 8-oxogeraniol in in vitro assays. We are currently investigating potential phosphatases for the formation of geraniol from GPP, potential iridoid synthases, and a number of oxidoreductases that may play a role in later steps of iridoid biosynthesis.

## Talk 14

### The dance of *Arabidopsis* with time, terrain, and tiny allies: Deciphering the microbiome-climate-plant triad

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Microbiota act as rapid responders to environmental cues, serving as indicators of ecological shifts with potential implications for host health. Understanding the effects of climate change on systems such as plants necessitates a comprehension of its impact on microbiome composition. Addressing this gap, we analyzed the phyllosphere microbiome assembly of the winter annual *Arabidopsis thaliana* across five distinct wild populations from 2019 to 2023, noting that only two of these growing seasons conformed to the historical norms of being colder and drier. By utilizing 16S-RNA/ITS amplicon sequencing and public climate data, we examined how assembly mechanisms might shape the climatic roles in diversity patterns. We found that fungal alpha and beta diversity in the phyllosphere were linked to a combination of spatial and temporal factors, with relatively minor effects from short-term climatic factors. This suggests a dominance of dispersal limitation in fungal community assembly, indicating a significant influence of long-term host and environmental factors. Conversely, bacteria displayed specific alpha- and beta-diversity patterns that were consistent across populations in 'normal' cooler years, highlighting a responsiveness to both short-term (alpha diversity) and long-term (beta diversity) environmental stress. This reflects the critical role of homogeneous selection in bacterial assembly, suggesting that selective processes are significantly shaped by climate. These findings illuminate how assembly mechanisms may underpin differential impacts of climatic and spatial factors on microbial diversity, underscoring the importance of considering both short-term and long-term environmental conditions to understand and predict the effects of climate change.



## Talk 15

### Olfactory signal guided behavior in *Locusta migratoria*

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Swarming locusts are major pests that threaten world's food production. Before swarm formation, locust populations exhibit a dramatic phase change from a solitary to a gregarious phase. The cause of this phase change is a complicated interplay of conspecific and environmental cues. This phase change in the migratory locust *Locusta migratoria*, still not well understood. Here we study the behavior of both solitary and gregarious *L. migratoria* towards the headspace odors of conspecifics. As we do not find a general attraction of gregarious animals to the headspace of gregarious conspecifics, swarm formation does not seem to be mainly governed by olfactory aggregation cues. When testing for potential mating signals, we observe that the headspace of virgin gregarious females is highly attractive only towards virgin males of the same phase, while mated gregarious males and solitary males, regardless of their mating state, do not become attracted. Interestingly, this phase-specific attraction goes along with the finding, that mating behavior in experiments with inter-phasic pairings is extremely rare. Our data suggest that odor emissions in *L. migratoria* play a significant role in a mating context.

## Talk 16

### External fungal symbionts increase performance of the Eurasian spruce bark beetle

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For over a century, it has been known that certain fungi living within the bark of Norway spruce, *Picea abies*, form tight associations with the Eurasian spruce bark beetle, *Ips typographus*. Recent evidence suggests that at least some of these associations qualify as true symbioses. Despite the insect's status as most economically important spruce pest in Europe, the ways in which fungi may contribute to bark beetle fitness have not been clarified. We are investigating whether one of these external fungal symbionts, *Grosmannia penicillata*, provides nutritional benefits to *I. typographus* bark beetles. Due to the lack of an artificial diet in which beetles will reproduce, we are rearing beetles in logs in laboratory experiments with and without this fungus. Our current results suggest that the exposure to *G. penicillata* has little effect on beetle reproduction until the second generation after initial inoculation. Chemical analyses of the fungus show that this symbiont is much richer in amino acids and B vitamins than the bark. The results will help clarify the nature of symbioses between bark beetles and their external fungal partners.

## Talk 17

### Plant diversity and community history shapes *Plantago lanceolata* metabolome

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Long-term biodiversity experiments have shown that plant diversity and productivity have a positive relationship that gets stronger over time. However, most studies have focused on productivity, with few on specialized plant metabolites. Phytochemical diversity is crucial for plant performance and defense. Environmental and genetic variation, herbivore and pathogen pressures, and resource availability can influence phytochemical richness and composition changes. Therefore, differences in plant species richness might affect plant metabolome composition by shaping different biotic and abiotic pressures. For the study, transplant experiments were done on *Plantago lanceolata* offspring to investigate the impact of plant-species richness on metabolomes and the influence of biodiversity selection pressures and community-shared history. We used seeds with biodiversity selection collected from plant communities of different diversity in a 17-year-old biodiversity experiment and planted in their origin environment and in communities with the same species composition, but "with soil history" or "without plant and soil history." Additionally, we compared the individuals grown in their original environment with the offspring of plants with no selection history (initial seed material). We analyzed leaf metabolomes (volatile and nonvolatile compounds) of *P. lanceolata* individuals in the field after one year of transplantation. We found that while the effect varied depending on the class of compounds studied, in general, we found that nonvolatile metabolome diversity increased with increasing plant diversity while volatile metabolome diversity decreased. Moreover, both biodiversity selection pressure and community history strengthen the plant diversity effect on plant metabolomes. Taken together, the present study shows that plant biodiversity and community history trigger unique responses in the plant metabolome. Furthermore, we found evidence of adaptive metabolomics responses to the different diversity environments. Understanding these dynamics can provide insights into how biodiversity influences plant chemistry and overall ecosystem functioning.

# Posters



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## Poster 1

### Gene clustering in barley reveals cryptic oxidative rearrangement in gramine biosynthesis

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Gramine is an alkaloid found in barley and other members of the Poaceae that provides protection against insects and affects their palatability to ruminants. The biochemical basis for the formation of gramine from the amino acid tryptophan has remained unresolved. Here we identify a gene cluster in barley containing two genes, encoding a previously reported *N*-methyltransferase as well as a cytochrome P450 monooxygenase, that we name AMI synthase (AMIS). We show that these two genes enable the production of gramine in the heterologous species *Nicotiana benthamiana*, *Arabidopsis thaliana* and *Saccharomyces cerevisiae*, and constitute the necessary genetic complementation to reactivate gramine biosynthesis in the barley variety Golden Promise that does not produce it. To further characterize *CYP76M57* in its natural host, we mutated the gene by Cas endonuclease technology in the cultivar Tafeno, which prevented the production of gramine in the plant. Based on *in vitro* experiments with yeast microsomes, we demonstrate that *CYP76M57* performs a cryptic oxidative rearrangement of tryptophan to an iminium intermediate. Taken together, our findings reveal how the gramine scaffold is generated from a simple amino acid. The discovery of the genetic basis of gramine formation enables access to breeding initiatives that aim to reduce pesticide use and harness the biological activity of gramine for barley cultivation.

## Poster 2

### Symbiont-symbiont interactions in a defensive symbiosis

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Symbiotic associations between insects and microbes are widespread and the benefits provided by the symbionts can be crucial for the survival of the host. Many insects only engage in mutualisms with particular bacterial species but a community of different taxa can also occur in a host. Although host-symbiont relations are often well studied, the interactions between different symbionts and their influence on the composition and functionality of a community remain poorly understood. Maintaining a symbiont homeostasis is important for keeping a symbiosis functional. Symbiotic functions comprise for example nutritional supplementation of poor diets, pheromone production or defense against antagonists.

In *Lagria villosa* beetles (Coleoptera: Tenebrionidae) we find such a defensive symbiosis in which bacteria protect beetle offspring from fungal infestation by producing different bioactive secondary metabolites. The microbial community of field-collected *L. villosa* is dominated by *Burkholderia gladioli*, but other bacteria like *Variovorax*, *Acinetobacter* and *Luteibacter* can also be found consistently across all life stages. Within *B. gladioli* we can find multiple symbiont strains. While *B. gladioli* Lv-StB is the most abundant strain in nature, it is lost after a few generations of lab rearing. *B. gladioli* Lv-StB has an eroded genome and cultivation attempts have not been successful so far. In contrast, *B. gladioli* Lv-StA is less abundant in nature but is culturable *in vitro*. To investigate the effect of co-occurrence of multiple *Burkholderia* strains on colonization success we utilize *in vivo* assays.

With our findings, we hope to contribute to the overall understanding of symbiont-symbiont interactions in multipartite symbiotic systems.

## Poster 3

### Mechanism of metabolic regulation in grain pest beetle-endosymbiont 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.

## Poster 4

### Immune division of labour in the clonal raider ant

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Social insects have evolved a wide range of strategies to combat the increased risk of disease outbreaks associated with group living. These strategies include group-level collective behavioural defences complementing individual-level physiological immune defences. However, the interplay between these two levels and its impact on disease spread remains largely unexplored. Here, we test the concept of "immune division of labour" within ant colonies by examining if individual immune activity is adjusted to the infection risk associated with different behavioural roles and assess how variation in immune activity may affect disease spread within the colony. We test this hypothesis in the clonal raider ant, whose unique biology enables us to quantify differences in immune investment among near-identical workers that nonetheless take on different behavioural roles. We quantify the relationship between individual behaviour (measured through automated tracking) and individual immune activity (measured through transcriptomics and pathogen exposure) and infer the epidemiological effects based on how the distribution of immune activity is linked to individuals' network position. The study sheds light on the fundamental relationship between immunity and behaviour and provides new insights into the epidemiological effects of heterogeneity in social groups.



## Poster 5

### Tissue-specific changes in young *Populus nigra* trees chemodiversity under *Lymantria dispar* herbivory

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The distribution and transport of metabolites among different plant organs is critical in defense against herbivorous insects, and this may be especially true in large woody plants. Here we studied black poplar (*Populus nigra*) and investigated metabolite profiles of four different tissues (*i.e.*, roots, wood, bark, young and old leaves) in trees after herbivory by the generalist-feeding caterpillar, *Lymantria dispar*. To do this, we analyzed tissue metabolites by targeted and non-targeted LC-MS/MS analysis in young, herbivore-damaged trees (n = 12). Our findings reveal notable tissue-specific variations in the concentration of specific metabolites. For example, salicinoids exhibit higher concentrations in leaves, particularly young leaves, while roots show greater sugar. Additionally, a cross-comparison of non-targeted results performed in two different laboratories to assess the reproducibility of these procedures revealed a consistent grouping of metabolites by tissue in both laboratories. These insights deepen our understanding of metabolomic responses in young black poplars under herbivory, providing valuable insights for future studies on plant defense mechanisms.

## Poster 6

### Imaging unimaginable symbiotic dynamics in a grain pest beetle

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The evolutionary success of many insects is attributed to mutualistic associations with microbes, which provide nutrition, defense compounds, and other natural products. Beetles are armored with a particularly thick and hard cuticle but not all diets provide sufficient amounts of the semi-essential amino acid tyrosine for cuticle formation. Thus, many beetles evolved symbiotic associations with symbiotic bacteria that complement dietary tyrosine via the shikimate pathway. Among grain pest beetles with tyrosine supplementary symbionts, *Prostephanus truncatus* harbors the ancient symbiont *Shikimatogenerans bostrichidophilus* that diverged into three lineages with complementary gene repertoires. Based on the symbiont genome assemblies, Fluorescence in situ hybridization (FISH) was used to localize bacterial lineage-specific DNA and RNA molecules. We resolved their 3D location within densely packed symbiont cells inside specialized insect organs via tissue clearing combined with highly resolved fluorescence microscopy, as well as volumetric electron microscopy. Imaging fluorescence-lifetime (FLIM) allowed multiplexing of FISH probes and counter staining while differentiating autofluorescence of insect tissues. In addition, after manipulating beetle diet and symbiont load, we measured the cuticle thickness and melanization of beetles using  $\mu$ CT scanning and an AI-supported pixel classifier. The symbiont genomic analyses showed complementary gene distributions among three bacterial lineages and implied the obligate metabolic exchange among symbionts. Various FISH results demonstrated life-stage-dependent dynamics of symbiont distribution and presumable interactions among symbiont cells via connections and transport. Lastly, experimental manipulation of symbionts resulted in thinner and brighter beetle cuticles, which verifies the functional integrity of metabolically fragmented symbiont genomes. The results suggest that *Prostephanus* necessarily need to maintain and transmit three lineages of symbionts, which is widely believed non-adaptive. Applying advanced imaging techniques, we depicted potential mechanisms of interactions among the interdependent bacteria. Quantification of the often co-localized signals and imaging of the presumably exchanged metabolites will be necessary to understand biochemical dynamics. Further, more studies on the biological dynamics of the symbiotic interactions and distribution in different beetle life stages are needed to elucidate the evolutionary driver of symbiont lineage splitting and their maintenance, which may be an overlooked phenomenon in other symbiosis systems due to the genomic similarities.

## Poster 7

### Evolutionary history of tyrosine-supplementing endosymbionts in pollen-feeding Dasytinae (Coleoptera: Melyridae) beetles

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Mutualistic relationships with bacteria can facilitate the colonization of ecological niches. However, detailed comparative studies of symbiont presence and function across host taxa are needed to unravel the evolutionary origins and dynamics of symbioses and their contributions to host fitness. Here, we investigated the evolutionary history and function of the symbiosis between pollen-feeding soft-winged flower beetles (Melyridae, Dasytinae) and their intracellular gamma-proteobacterial partner '*Candidatus Dasytiphilus stammeri*'. Reconstructing the distribution of the symbiont within the Dasytinae phylogeny unraveled a long-term coevolution, originating from a single acquisition event with subsequent host-symbiont codiversification, but also several independent symbiont losses. The analysis of 20 different symbiont genomes revealed that their genomes are severely eroded. However, the universally retained shikimate pathway indicates that the core metabolic contribution to their hosts is the provision of tyrosine for cuticle sclerotization and melanization. Despite the high degree of similarity in gene content and order across symbiont strains, the capacity to synthesize additional essential amino acids and vitamins and to recycle urea is retained in some but not all symbionts, suggesting ecological differences among host lineages. This report of tyrosine-provisioning symbionts in pollen-feeding insects expands our understanding of tyrosine supplementation as an important symbiont-provided benefit across a broad range of insects with diverse feeding ecologies.

## Poster 8

### Life with long appendages: Functional, kinetic and morphological adaptations in house centipedes

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The house centipede, *Scutigera coleoptrata*, is known as one of the fastest invertebrate predators. Although not too welcome inside homes, these centipedes are regarded as excellent biological pest control around vineyards. Endemic to the mediterranean region, this species is nowadays also common throughout central Europe. Scutigermorph centipedes are generally characterized by extremely 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. Most outstanding, however, is the functional transformation of the ultimate legs. They are not involved in locomotion or prey capture, are much longer and have a much higher number of sensory structures than normal locomotory legs. Moreover, their position and kinematics greatly resemble those of the antennae. Thus, it is assumed that they have a predominantly sensory function. Focusing on mechanoreception and olfaction, we aim to better understand the neurological and functional aspects of scutigermorph appendages, using the house centipede as a model. By means of electrophysiology, antennal and leg backfills, as well as immunohistochemistry and morphological investigations we want to determine to which extent the ultimate legs act as “posterior antennae”, and how the structure of the ultimate ganglion correlates to this function. Furthermore, we aim to understand how these animals coordinate the 14 pairs of elongated legs involved in locomotion and hunting. High-speed video recordings, as well as step-force measurements will reveal the kinetic mechanism they evolved in order to achieve their exceptional speed and predatorial success.

## Poster 9

### Genetic determination of color polymorphisms in grasshoppers

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Polymorphism is the occurrence of two or more different morphs in the same population of a species. Color polymorphism, as one of visible “marker phenotypes”, has attracted a lot of interests from scientists for a long time. Scientists try to explain the fascinating diversity with a theoretical framework of “balance selection”, which consists of negative frequency-dependent selection, heterozygote advantage and spatial environmental heterogeneity. The relative strength of these forces and the way they interact with each other depend not only on the environmental conditions, but also on the underlying determination mechanisms of polymorphism. Therefore, parsing the genetic basis could be highly conducive to understand how polymorphism forms.

Orthoptera, represented by grasshopper and bush cricket, exhibits a wide-spread green-brown color polymorphism in nearly one third of species. The highly prevalent body color dimorphism and rich biological resources make orthoptera an ideal research model to explore the ecological context, regulatory mechanism, and evolution of polymorphism. Our team’s previous research on club-legged grasshopper *Gomphocerus sibiricus* has indicated absence of environmental determination on coloration and provided evidence for genetic basis of green/brown morph at a single autosomal locus. Differential expression analysis of *G. sibiricus* transcriptome data revealed upregulated expression of beta-carotin binding protein ( $\beta$ CBP) in green morph relative to brown morph. This discovery inspired us to propose a hypothesis that green color in *G. sibiricus* could be led by the combination of blue pigment with yellow complex of beta-carotin and  $\beta$ CBP. In contrast, previous research of a swarming species *Locusta migratoria* proposed a totally distinct coloration strategy, where highly expressed  $\beta$ CBP leads to brown morph instead of green.

In this study, we will combine the analysis on transcriptome, genome, and QTL mapping data of two grasshopper species from Gomphocerinae subfamily, i.e., *G. sibiricus* and *Pseudochrothippus parallelus*, to elucidate their coloration regulatory pathways. We also plan to verify the function of putative coloration genes by RNA interference (RNAi) experiment. Finally, we will compare the coloration strategy in Gomphocerinae with that discovered in *L. migratoria* and infer the evolution of green-brown coloration mechanism in grasshoppers occupying diverse habitats.

## Poster 10

### Nematode infections reshape ant chemical signatures

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Social insects are attractive hosts for parasites because they live in dense colonies where related hosts engage in frequent social interactions. Parasites can also exploit and manipulate various host behaviors to their advantage. However, behavior in most social insect colonies often covaries with individual traits like age and genotype, thereby leaving the mechanisms and implications of parasite-driven behavioral changes largely unexplored.

Nematodes of the genus *Diploscapter* naturally infect the heads of ants and have been shown to affect behavior in the clonal raider ant (*Ooceraea biroi*). These ant colonies are queenless; all workers reproduce asexually and synchronously, providing experimental control over age and genotype at the individual and group levels. Intriguingly, the nematodes infect a specific organ in the head of ants, the pharyngeal gland (PG). The PG is involved in the storage and spread of cuticular hydrocarbons (CHCs), which themselves form the basis for nestmate recognition. This raises questions about whether and how nematode infections in the PG alter the host CHC profiles and nestmate recognition behavior. To address these questions, we first analyzed the CHC profiles of age-matched infected and uninfected ants from five asexual genetic lineages of clonal raider ants using thermal desorption GC-MS. We then used controlled behavioral assays to measure the effects of the observed chemical changes on nestmate recognition. This work serves as a starting point to explore the implications of infection-mediated chemical changes on host social structure and parasite transmission.

## Poster 11

### Elucidating the biosynthetic pathway of *Cinchona* alkaloids: Identification of a dihydrocorynantheol synthase

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*Cinchona* alkaloids encompass four principal compounds: quinine, quinidine, cinchonidine, and cinchonine, constituting a distinctive class of quinoline alkaloids renowned for their numerous applications in medicine and chemical synthesis. Among these, quinine stands out as the flagship molecule due to its remarkable antimalarial properties. Despite the considerable advancements in the total chemical synthesis and analysis of *Cinchona* alkaloids, the biosynthetic pathways within *Cinchona* species remain largely elusive. Feeding studies with radiolabeled substrates have shown that these compounds belong to the monoterpene indole alkaloids (MIAs) family, deriving from a series of transformations of the common precursor, strictosidine. Recent investigations from our group have identified the enzymes converting strictosidine to dihydrocorynantheal, an advanced intermediate en route to the abovementioned *Cinchona* quinoline alkaloids. As part of our efforts to unveil downstream processes and involved enzymes, we discovered an aldo-keto reductase (AKR) that efficiently converts dihydrocorynantheal into dihydrocorynantheol, another known metabolite from *Cinchona* plants and related species. Other AKRs yielding a yet structurally unidentified genuine *Cinchona* metabolite were also identified.

## Poster 12

### Understanding alkaloid biosynthesis in ladybird beetles

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Alkaloids are nitrogen-containing organic substances of natural origin widely distributed in plants, fungi, bacteria, and insects. While the biosynthesis of alkaloids in plants has been intensively studied, little is known about how insects produce these compounds. Ladybirds (Coccinellidae), collectively synthesize 50 structurally diverse alkaloids, including acyclic amines, piperidines, pyrrolidines, azamacrolides, and homotropanes, that protect them from predation. This project aims to study how alkaloids are produced in two ladybird species, namely *Propylea quatuordecimpunctata* and *Adalia bipunctata*. Feeding isotopically labelled precursors to several species of ladybirds showed that many of these alkaloids share a common biosynthetic origin via a fatty acid precursor. This common origin will streamline pathway discovery. Moreover, *in vitro* assays with crude insect extracts suggest that alkaloid biosynthesis takes place in the fat body. By comparing the transcriptome of the fat body and other tissues, we identified candidate genes potentially involved in alkaloid biosynthesis. The next steps will require the expression of candidate genes in heterologous hosts and *in vitro* enzyme assays. RNAi silencing of these candidates in the corresponding species and measuring the resulting alkaloid levels by GC-MS will help us to identify biosynthetic genes *in vivo* and probe the biological function of these alkaloids.



## Poster 13

### The role of *R. insecticola* in pea aphid performance and plant defense response

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The native legume pea aphid, *Acyrtosiphon pisum*, consists of at least 15 genetically distinct biotypes, each adapted to only one or a very few host plants. However, all biotypes can also perform well on the universal host plant *Vicia faba*. Like most insects, the pea aphids host endosymbionts. The obligate endosymbiont *Buchnera aphidicola* provides the aphid with essential amino acids not present in the phloem sap. Many pea aphids also harbor one or sometimes two facultative endosymbionts, the role of which is often unknown or under investigation.

The pea aphid biotype, native to *Trifolium pratense*, is predominantly associated with the facultative endosymbiont *Regiella insecticola*. *R. insecticola* is known to confer protection against entomopathogenic fungi but the reason for the predominant association with the *Trifolium* biotype pea aphids is unknown. Therefore, we investigated whether pea aphids of the *Trifolium* biotype differ in their performance depending on whether they harbor *R. insecticola* or not. While the performance of one pea aphid clone on its native host plant *T. pratense* was not strongly affected by the presence of *R. insecticola*, the corresponding *R. insecticola*-carrying pea aphid clone showed a reduced fecundity and higher mortality on the universal host plant *V. faba*. We investigated whether this was due to a stronger induced defense response or changes in metabolic response of plants.

We examined the plant phytohormone, amino acids, and metabolic response to pea aphids with and without *R. insecticola* to see if plant responses were responsible for the difference in aphid performance. However, the plant defense response towards aphids with and without *R. insecticola* differed only slightly. In *V. faba*, there was an increase in salicylic acid (SA) and its glucose conjugate (SA-Glu) upon aphid feeding. The levels of jasmonic acid-isoleucine (JA-Ile) slightly increased when aphid with endosymbiont was feeding while its hydroxyl conjugates (OH-JA-Ile) slightly decreased upon aphid feeding in both the hosts. The aphid's endosymbiont composition had no significant effect on most of the phytohormone levels except SA. We further examined the metabolome to check for induced secondary metabolites in response to aphid feeding. The metabolome of both the hosts was significantly different in response to aphids with and without *R. insecticola*. Thus, host plant metabolites may play an important role in determining the performance of the aphid on different hosts.

## Poster 14

### Untargeted metabolomics with electron ionization: A roadmap for adapting SIRIUS and beyond to GC-EI mass spectrometry data

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Tandem mass spectrometry (MS/MS) measurements are a preferred choice for untargeted metabolomics experiments. Using soft ionization techniques like electrospray ionization (ESI), they keep the molecular ion intact for measurements of its mass-to-charge ratio before further fragmentation. Methods like SIRIUS use this property when generating a molecular formula from a spectrum by only considering formulas fitting the molecular ion's mass. Experiments using electron ionization (EI) often do not offer this kind of information, as the hard ionization fragments the molecule more strongly and can lead to no molecular ion staying intact. However, EI can ionize different molecules, produce highly reproducible spectra, and is often coupled with gas chromatography (GC), the most commonly employed separation technique in metabolomics which has been in use for several decades. Here, we outline a roadmap on what challenges must be overcome to adapt methods like SIRIUS to GC-EI-MS spectra and what benefits this can bring. First, missing molecular ion peaks need to be identified and their informational void is to be filled, for instance by employing combinatorics or machine learning to reconstruct missing peaks. Once this is achieved, SIRIUS benefits from isotope patterns in EI spectra due to its fragmentation tree approach, but interfering radical losses of H and H<sub>3</sub> have to be accounted for. After SIRIUS can handle EI spectra, machine learning methods integrated into SIRIUS can be adjusted and newly trained on data from this kind of spectra; this includes CSI:FingerID for searching structure databases, and MSNovelist for generating molecular structures. Unfortunately, few data are publicly available for high-resolution EI spectra from standards. This impedes the training of the machine learning methods, which require high mass accuracy of measurements. To this end, we want to "lift" abundantly available low-resolution EI spectra to high resolution and high mass accuracy. We are confident that the additional structural information from fragment-rich EI spectra may result in a strong predictive power of the aforementioned machine learning methods.

## Poster 15

### Molecular basis and specificity of symbiont colonization in *Lagria villosa*

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The benefits incurred by microbial symbionts are lucrative for many animals. In the beetle species *Lagria villosa*, vertically transmitted bacterial symbionts (chiefly *Burkholderia*) protect eggs and larvae against fungal pathogens. The system is peculiar in that the larvae house the symbionts in external cuticular invaginations. However, little is known of the molecular factors involved in the colonization of the external symbiotic organs. Therefore, this project will seek to unearth the colonization factors essential for *Burkholderia* establishment in beetle and plant hosts. Furthermore, the colonization specificity and the host's ability to regulate the bacterial community in these organs will be assessed. Lastly, the natural variation in the chemical defenses between different *Lagria* beetle populations, species, and how this relates to the composition of their symbiotic community, will be investigated. Through this study, we hope to gain insight into the molecular mechanisms underlying symbiont colonization and partner specificity in a ectosymbiosis involving specialized cuticular organs. Finally, the determination of colonization specificity and how chemical variation relates to the bacterial community could provide interesting insights into the dynamics and relevance of defensive symbioses under natural conditions.

## Poster 16

### Are you sick? - The chemical and sensory bases of social immunity in the clonal raider ant

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Social insects live in densely populated colonies, leading to an increased risk of disease outbreaks. Consequently, they have evolved collective disease defense strategies known as 'social immunity', wherein nestmates cooperate to combat disease. For example, social insects groom infectious fungal spores off each other's body surface, thereby preventing infection. Social immunity relies on the ability to detect sick individuals 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*, which affords control over age and genotype at the individual and group levels. This blind species relies heavily on chemical communication, which makes the detection of sick nestmates by olfactory cues likely. Our objectives are to 1) identify "disease cues" by comprehensively screening the chemical profiles of immune-challenged and infected ants, 2) characterize the behavioral responses towards workers infected with different pathogens, followed by testing the previously identified chemicals, and 3) investigate how the response to these "disease cues" is encoded in the brain through in vivo calcium imaging of the antennal lobe.

By using a combination of chemical, neuronal, and behavioral analysis, we anticipate shedding light on the chemical and sensory bases of social immunity in insect societies.

## Poster 17

### Mating experience changes the oviposition preference of *Drosophila melanogaster*

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Insects exhibit behavioral changes based on their experiences. It is known that the experience of copulation has a profound effect on the behavior of male *Drosophila melanogaster*, e.g., males perceive ejaculation as a reward and increase their preference for odors they smelled during ejaculation. Courtship conditioning, hence, is a well-established paradigm that however uses primarily male behavior. Previously, we have shown that experiences during oviposition significantly change the oviposition preference of female flies. However, it is not known whether female flies regard mating as rewarding also and are able to learn cues during mating that can influence subsequent behaviors, such as oviposition or remating preference. We hypothesized that *D. melanogaster* females would prefer to oviposit on those substrates they have mated on. Indeed, when females were mated on a particular substrate, their subsequent oviposition preference for that substrate increased significantly. Moreover, when females were either anosmic or during mating experienced only the headspace of the substrate, they did not change their oviposition preference upon mating. Apparently, olfactory input is needed but not sufficient for the mating-induced change of preference.

Ongoing experiments shall reveal whether the mating experience changes not only the female's oviposition preference but also its mating preference when choosing another male later.

## Poster 18

### Individual and social immunity in the clonal raider ant

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To counteract the increased risk of disease outbreaks that is associated with group living, ants and other social insects deploy not only physiological immune defenses acting at the individual level but also collective behavioral defenses acting at the group level. Nevertheless, whether and how physiological immune defenses and behavioral defenses interact is not fully understood.

To address this gap, we use the clonal raider ant *Ooceraea biroi* and the entomopathogenic fungus *Beauveria bassiana* as a study system. Clonal raider ant colonies lack queens but consist of workers that reproduce clonally and synchronously. This unique biology provides experimental control over several factors (age, genotype) known to affect both behavior and immune responses at the individual and group levels. Like other ants, *O. biroi* grooms nestmates exposed to fungal spores, effectively removing fungal spores from their cuticle, and enhancing their survival. Here, we first characterize individual immune responses to *Beauveria* exposure using RT-qPCR. We then combine behavioral and molecular analyses to ask whether allogrooming modulates individual immune responses. Finally, we ask whether individual and social immunity are modulated by the social context by measuring immune responses and allogrooming in colonies in which only some workers were exposed to fungal spores. This work improves our understanding of the clonal raider ant's immune system and starts to elucidate the interaction between physiological immune defenses and social immunity.

## Poster 19

### Investigating the costs and benefits of a host plant-dependent chemical defense in a herbivorous insect

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Chemical defense compounds of plants play an important role in ecological networks by shaping interactions with herbivorous insects and their natural enemies. One mechanism through which plant defense compounds shape multitrophic interactions is sequestration, the accumulation of plant defense compounds in the insect for protection from predators. We use the horseradish flea beetle (*Phyllotreta armoraciae*) as a model to understand the molecular basis and ecological consequences of insect sequestration strategies. This specialist herbivore sequesters glucosinolates from its brassicaceous host plants and uses endogenous myrosinase enzymes to convert them to toxic isothiocyanates for defense. We discovered that *P. armoraciae* possesses three different myrosinase genes with distinct ontogenetic expression patterns. These genes encode enzymes with different substrate preferences, which likely represent an adaptation to the divergent glucosinolate profiles of above- and belowground plant tissues that *Phyllotreta* adults and larvae use as a food source, respectively (Körnig et al., 2023).

Interestingly, we additionally discovered natural genetic variation of myrosinase activity in *P. armoraciae*. Through haplotype-resolved genome sequencing, we found that the number of myrosinase genes encoded in the beetle genome varies between individuals. This gene copy number polymorphism influences the level of myrosinase activity in larvae but not in adults. The selection of beetle lines with larvae having high or low myrosinase activity allows us to investigate the costs and benefits associated with sequestered chemical defenses. For example, physiological costs of higher myrosinase activity may result from the detoxification of reactive isothiocyanates while having the benefit of a better protection against predators. To investigate possible costs, we currently compare several fitness parameters of beetles with high and low myrosinase activity under controlled conditions. To investigate benefits of this chemical defense, we determine the impact of high and low myrosinase activity on the ability to deter entomopathogenic nematodes, which are potential biological control agents of flea beetle pests in agricultural ecosystems. We hope that our results will contribute to the development of sustainable pest control strategies for flea beetle pests and to understanding how plant chemical defenses shape trophic networks.

## Poster 20

### Host control in symbiont cell morphology: decoding the intricacies of beewolf wasp's symbiont transmission

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Beewolves wasps have established a symbiotic association with *Streptomyces* bacteria that are cultivated in the antennal gland reservoirs of female wasps. The symbionts produce a complex mixture of different antibiotics that protect beewolf larvae during the long period of hibernation. Throughout 68 million years of evolutionary history of the association, both partners have undergone specialized adaptations to maintain and secure the symbiosis across generations.

To understand which genes are involved in the host-symbiont interaction, we assessed gene expression profiles in the antennal segments of aposymbiotic (apo) and symbiotic females. In the antennal gland reservoirs of beewolves, we identified host candidate genes annotated for functions related to maintaining immune homeostasis, nutrient supplementation, and transporters. Among these genes, we found an upregulated C (chicken) type lysozyme gene. Beewolf symbionts typically grow inside the antennae as short hyphal fragments. However, when grown in liquid culture, they adopt a free-living *Streptomyces*-like growth pattern, forming long branched filaments and colonies. Interestingly, when liquid media is supplemented with C-type lysozyme, the bacteria exhibit a change in cell phenotype, resembling the shortened cells observed in the host gland reservoirs. This alteration in cell phenotype *in vivo* could be crucial for the effective secretion of symbiotic cells and their transmission to new generations.

Thus, the host C-type lysozyme likely plays a critical role in controlling the cell shape of the symbiont, contributing to the successful secretion of symbiotic cells from the antennae of females before oviposition.



## Poster 21

### ***Arabidopsis* foliar bacteriome under simulated winter**

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The phenotypic plasticity of *Arabidopsis thaliana* facilitates adaptation to diverse environmental conditions, including the challenges posed by winter. As global climate change increases the unpredictability of winter weather, understanding its impact on the *A. thaliana* bacteriome becomes essential. Despite its significance, there is a notable lack of research on how winter conditions affect microbial dynamics on plant leaves. This study aims to elucidate the effects of simulated winter conditions on the leaf bacteriome of *A. thaliana*, with a focus on the influences of genotype and environmental stressors. By comparing the bacteriome under winter-simulated conditions to that under standard laboratory settings, we investigate the adaptability of *A. thaliana* through both genotypic and hormonal pathway lenses. Select ecotypes of *A. thaliana*, representing diverse geographical origins, were co-cultivated under controlled simulated winter conditions. Additionally, Columbia mutants with disrupted hormonal pathways (Abscisic acid, Ethylene, and Salicylic acid) were analyzed to determine the pathways' roles in winter adaptation. High-throughput amplicon sequencing was utilized to profile the leaf bacteriome across varying conditions. We hypothesize that exposure to simulated winter conditions results in altered bacteriome compositions on *A. thaliana* leaves compared to those grown under standard conditions. We expect that variations in winter severity will produce distinguishable shifts in the leaf bacteriome, with ecotypes from different geographical origins showing unique responses reflective of their native adaptation mechanisms. Furthermore, we anticipate that disruption of key hormonal pathways will significantly impact the bacteriome composition, highlighting their importance in the plant's winter response. This research is poised to advance our understanding of the complex interactions between plant genotypes, hormonal regulation, and environmental stress factors. By revealing how native environmental adaptations and hormonal signaling pathways influence microbiome dynamics under simulated stress, the study will provide insights into the resilience mechanisms of *A. thaliana* in the face of changing winter climates.