Public Understanding of Life Sciences / Chemical Ecology

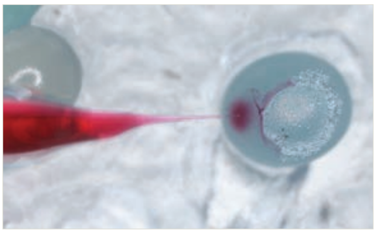
PULS/CE 34

Max Planck Institute for Chemical Ecology

Newsletter November 2019

Using skin to perceive color

Twig-mimicking caterpillars recognize the color of the background with their skin and either change their own skin accordingly. In these animals, genes that are required for vision are expressed not only in their eyes but also in their skin. They use this mimicry to protect themselves from predation ... **p. 3**



Genomic editing of a moth's olfaction

CRISPR/Cas9 provides a new tool to analyze moth olfaction and study interactions between insects and plants. Scientists demonstrated in behavioral studies that foraging is visibly disturbed in moths with a dysfunctional sense of smell, although these moths are still able to locate plants on which to lay eggs ... **p. 4**

Context matters

Environmental conditions and the genetic make-up of individual plants influence insects' responses to the plant odor linalool. Researchers identified the gene which controls linalool production and which is responsible for variable linalool emissions in different plants of the same species ... **p. 5**

MAX-PLANCK-GESELLSCHAFT

Research with tradition

Axel Mithöfer pointing to Stahl's memorial plaque in the lecture hall of FSU's Matthias Schleiden Institute: Stahl was appointed chair of botany in 1881. Until his death, he was also the director of the botanical garden. *Photo: Angela Overmeyer, MPI-CE*

The Long Night of the Sciences program includes the lecture about Stahl on November 22, 2019, which will be offered at 7, 8, and 9 p.m. in our Ernst Stahl Room.

Dear Readers!

On December 3, 2019, almost exactly 100 years ago, botanist Christian Ernst Stahl (1848-1919) died in Jena. In 1881, Stahl was appointed professor and head of the botanical institute as well as director of the botanical garden of the University of Jena. In the course of his scientific career, he devoted himself to various areas of research, from plant sciences to ecological questions.

The term "ecology" had been introduced to the sciences by his colleague at Jena University, zoologist and evolutionary biologist Ernst Haeckel although Haeckel himself never conducted any ecological studies. Considered the founder of experimental ecology and ecophysiology, Stahl combined observations in the field with lab experiments, and studied the influence of different environmental factors on plant development.

Of particular importance for today's research is his discovery that plants produce chemical substances as defenses against herbivory. He realized that various plant substances - alkaloids, tannins, terpenes, and many other secondary plant metabolites - played a defensive role.

He discovered that the biosynthesis of such plant protective substances was the result of Darwinian selection. Although his scientific legacy was forgotten for decades, Christian Ernst Stahl can truly be considered a pioneer and co-founder of our field of expertise: chemical ecology.

Given Stahl's history as well as the focus of our institute, Jena is the ideal place to study phytochemicals as well as their biosynthesis and effects. Since 2019, this research area has been considerably strengthened by the appointment of director Prof. Sarah O'Connor to lead the new Department of Natural Product Biosynthesis. On pages 6 and 7 of this newsletter, Sarah will tell us more about research projects in her department and the plans she has for the coming years.

The Long Night of the Sciences in Jena, which will take place this year on November 22, 2019, offers a special opportunity to take a look at Stahl's pioneering research in the field of plant chemical ecology. Axel Mithöfer, head of the Research Group Plant Defense Physiology, will present Stahl's scientific achievements and discuss his forgotten impact on chemical ecology.

Axel Mithöfer and Angela Overmeyer

Using skin to perceive color

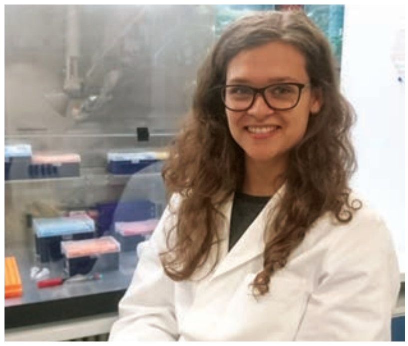
Cephalopods, chameleons and some fish camouflage themselves by adapting their color to their surroundings. These animals are able to perceive color and light independently of the eyes. Some insects, such as caterpillars of the peppered moth, also match their body color to the twig color of their food plant, although this color change is rather slow compared to in other animals.

In a new study, researchers from Liverpool University and the Max Planck Research Group Predators and Toxic Prey pursued three different approaches before finally solving the riddle of how caterpillars of the peppered moth match the color of their surroundings.

First, they tested if caterpillars of the peppered moth, whose eyes had been painted over with black acrylic paint, could after being "blindfolded" adjust their color to the background. These blindfolded caterpillars were raised on white, green, brown and black branches, and their body color observed. Even without being able to see, the caterpillars changed color to resemble their background just like caterpillars whose eyes had not been covered. In behavioral experiments, blindfolded caterpillars had the choice to move to differently colored twigs. Consistently, these caterpillars rested on the twig whose color was most similar to their own.

The researchers also examined caterpillars' bodies to discover where genes related to vision were expressed. The genes were found not only in the head of the caterpillars, where the eyes are, but also in the skin of all body segments. One visual gene was expressed even more in the skin than in the heads of the caterpillars. This gene is probably involved in the perception of background color by the skin.

One of the major challenges animals face is how to avoid being eaten by predators. Numerous species have evolved camouflage to avoid being detected or recognized. Color change enables animals to match their surroundings and potentially reduce the risk of predation. The computer model the scientists constructed, which can 'see' the same way birds do, enabled them to conclude that these adaptations - color change, twig-mimicry, behavioral background-matching - likely evolved to avoid visual detection by predators. Caterpillars with better color sensing may have been eaten less often by birds, even as birds with improved vision may have been preyed more upon these larvae, continuing the evolutionary predator-prey arms race. [KG]

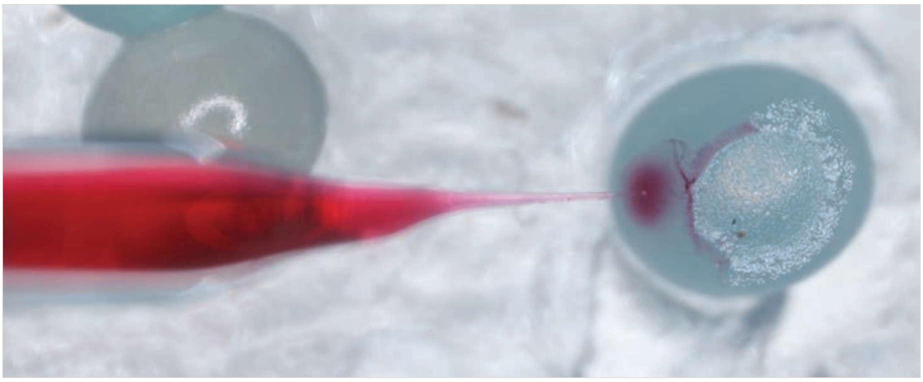


Above: Amy Eacock, first author of the study, is a postdoc in the Max Planck Research Group Predators and Toxic Prey, led by Hannah Rowland. *Photo: MPI-CE*

On the left and below: Caterpillars of the peppered moth (*Biston betularia*) sense color through their skin and match their body color to the background to protect themselves from predators. Even blindfolded caterpillars are able to sense colors. The study expands our understanding of how lepidopteran larvae protect themselves from predation. *Photos: Arjen van't Hof, Universität Liverpool.*

Original Publication: Eacock, A., Rowland, H. M., van't Hof, A. E., Yung, C. J., Edmonds, N., Saccheri, I. J. (2019). Adaptive colour change and background choice behaviour in peppered moth caterpillars is mediated by extraocular photoreception. **Communications Biology**, 2: 286

Genomic editing of a moth's olfaction



The CRISPR/Cas9 revolution in ecological research: CRISPR/Cas9 gene editing allows for a targeted inactivation of genes. The Cas9 enzyme acts as molecular scissors for DNA, and a single-stranded guide RNA binds to the Cas9 enzyme and directs it to a specific location in the region of interest, in this case, the Orco gene. Richard Fandino developed a technique that allowed molecular components that had been dissolved in a buffer solution (dyed red) with a quartz glass capillary to be injected into the sphingid egg without damaging the egg or the developing tobacco hawkmoth embryo. *Photo: Richard Fandino, MPI-CE*

Original Publication:

Fandino, R. A., et al. (2019). Mutagenesis of odorant co-receptor Orco fully disrupts foraging but not oviposition behaviors in the hawkmoth *Manduca sexta*. **PNAS**, 116 (31) 15677-15685

The study of ecological interactions between plants and insects which are mediated by chemical signals is a topic of central interest in the field of chemical ecology. Insects are the recipients of plant signals. What role does their sense of smell play in processing those signals? A team of scientists led by Richard Fandino from the Department of Evolutionary Neuroethology has now explored this question experimentally. The researchers applied CRISPR/Cas9 in the tobacco hawkmoth *Manduca sexta* for a targeted gene modification. The target of the CRISPR/Cas9 molecular scissors was the gene that codes for the co-receptor Orco. The team discovered that this protein plays a crucial role in insect olfaction, as CRISPR-Cas9 moths had severe behavioral deficits. Comparative studies between moths carrying this mutation and moths whose sense of smell is intact provide invaluable information about the importance of olfactory receptors.

The researchers observed that foraging moths without a functional co-receptor Orco landed abruptly on the flower of their food plants, rather than first hovering in front of the flower and extending their proboscis. Although most genetically modified moths can still find flowers and plants when foraging, they only land on the flower instead of drinking nectar. Tests of ovipositing moths, however, showed that differences between wild-type moths and moths without Orco were less significant than differences observed in modified moths that were foraging. More than half of the modified gravid insects exhibited the hawkmoth's characteristic olfactory-directed flight towards plants selected for ovipositing and then laid their eggs.

Insect olfaction is based on different receptor types. Apart from olfactory receptors, there are also the evolutionarily much older ionotropic receptors, which are also involved in detecting many odors. The ionotropic receptors can be considered the "ancient nose" of all arthropods. The two "noses" may explain why moths without Orco are not completely anosmic (unable to perceive smells): The "ancient nose" can also also detect chemosensory plant cues and these are sufficient for locating a host. The more modern "insect nose", on the other hand, detects olfactory cues that provide more qualitative information about its host. Therefore, floral foraging and pollination behaviors rely heavily on the insect nose.

The goal of this research is to better understand insect olfaction and its role in the interaction with the processing of further sensory signals, such as the visual cues of flowers and leaves of a plant, but also humidity or CO_2. Such insights are important in order to ecologically reconcile the protection of beneficial insects, such as pollinators, with the control of plant herbivores. [AO/KG]

Context matters

Plants have evolved multiple strategies to defend themselves against herbivorous animals, especially insects. In addition to mechanical defenses, such as thorns and spines, plants also produce chemical defense compounds that hold insects and other herbivores at bay. These substances include volatile organic compounds, often produced by plants only after insect attack. Linalool is such a plant volatile organic compound; it mediates different ecological interactions with insects. It is known that linalool in tobacco plants can attract predatory *Geocoris* bugs to show them the way to their prey: the eggs or freshly hatched larvae of tobacco hawkmoths. However, as a floral scent component, linalool is also attractive for adult hawkmoths and influences the decisions of mated female moths to lay their eggs on a plant.

A team of scientists from the Department of Molecular Ecology led by Meredith Schuman and Ian Baldwin has now studied the ecological functions of the monoterpene linalool in wild *Nicotiana attenuata* plants in more detail. The researchers observed a correlation between the rate of *Manduca sexta* eggs predated by *Geocoris* bugs and the amount of linalool produced by the respective plants. Such a correlation was not, however, observed between five similar organic compounds emitted by tobacco plants and the egg predation rate. This discrepancy indicates that the emission of linalool functions as the plants' chemical cry for help, attracting predatory bugs that in turn attack herbivorous larvae.

However, tobacco plants vary a lot in their linalool emission. Experiments with plants whose ability to produce linalool had been genetically modified revealed that for ovipositing moths, attractiveness as an oviposition site is related to a plant's genetic background. But the differences in the moths' responses to linalool emission vanished as the surrounding environment became more complex.

The study illustrates that moths pay attention to many different features of plants when choosing where to feed or oviposit. They integrate this information with what they already know in order to choose among the available plants. Thus, the availability of alternative plants and their characteristics are likely to determine the importance of any individual cue: in this case, linalool.

A better understanding of context-appropriate plant defenses against herbivores might help workers in standardized industrial agriculture overcome problems such as the evolution of resistance to commonly used pesticides. [AO/KG]

Female hawkmoths (*Mandua sexta*) prefer to lay their eggs on plants with a naturally high linalool production. At the same time, increased linalool emission results in more eggs, and freshly hatched *Manduca* larvae becoming the prey of predatory *Geocoris* bugs. Behavioral assays in increasingly complex environments showed that the effects of linalool are quite variable, depending on the natural environment and the genetic makeup of the plant. *Photo: Danny Kessler, MPI-CE*

Original Publication: He, J., et al. (2019). An unbiased approach elucidates variation in (*S*)-(+)-linalool, a context-specific mediator of a tri-trophic interaction in wild tobacco. **PNAS** 116 (29) 14651-14660

Cats are attracted by the smell of catmint (*Nepeta mussinii*). Plant odors, so-called nepetalactones, are responsible for the fact that the plant acts as a cat attractant. Moreover, nepetalactones, which belong to the irodoids, are insect-repellent. O'Connor and researchers in her department study how plant biosynthesize these substances. *Photo: Phil Robinson, John Innes Centre*

Recent Publikations:

Lichman, B. R., et al. (2019). Uncoupled activation and cyclization in catmint reductive terpenoid biosynthesis. **Nature Chemical Biology**, 15, 71-79

Caputi, L., et al. (2018). Missing enzymes in the biosynthesis of the anticancer drug vinblastine in Madagascar periwinkle. **Science**, 360(6394), 1235-1238

Nature as a chemist

Last year, Sarah O'Connor was appointed scientific member of the Max Planck Society and head of the new Department of Natural Product Biosynthesis. This means that for the first time, our institute has a female director on board. Meanwhile, her lab has moved from the John Innes Centre in Norwich, United Kingdom, to Jena. Most boxes have been unpacked. However, many alterations remain to be completed before the new department is fully functional. Therefore, we are very glad that Sarah still took her time to answer our questions:

Did you always want to become a chemist?

Unfortunately, I have no inspirational story to tell. I didn't blow up the basement when I was a kid with a chemical explosion or anything like that. My grandfather and father worked as chemists, and I think that made chemistry seem very familiar to me. I got good grades in science in school and so at University I decided to major in chemistry without thinking about it too much. But I chose the University of Chicago because this school requires that all students take many literature, history and art courses. So, if I changed my mind about science, it would be easy for me to switch to literature, which was another subject that I loved. But once I started working in a research lab as an undergraduate researcher, I was hooked. I knew I wanted to do research.

What aspect of natural product research do you find especially fascinating?

As an organic chemist, enzymes have fascinated me since I started my PhD. I want to know how an enzyme can evolve to carry out very complicated and specific chemical reactions, and I want to discover the factors or the conditions in a plant that allow this to happen.

What are you currently studying and which research projects did you bring to Jena?

We brought all of our research projects to Jena. We work on two major plant systems, the Apocynaecea that make alkaloids (like vinblastine) and the Lamiaceae that make iridoids, which are a type of monoterpene known to have lots of interesting ecological roles. We have done a lot of work to map out many of the biosynthetic pathways in these plants, and there is still so much more interesting chemistry to be discovered. But now at Jena we can take this a step further and start looking at how these same pathways behave in the plant. We know surprisingly little about the function of these molecules and how these molecules evolved in the plants.

What has been your most exciting discovery so far?

I am excited by all of our discoveries! But, we have been working to understand how the plant Catharanthus roseus makes the anti-cancer drug vinblastine for a very long time. Last year, after 15 years, we finally reported the 4 last enzymes in this pathway. Part of why this discovery was so exciting was because it explained how a plant catalyzes a complicated chemical reaction called a Diels-Alder, thought to be rare in nature. Our work showed how the plant synthesizes a starting material that one enzyme can convert into one product via one type of Diels-Alder reaction, and a second enzyme can use this same starting material to catalyze a different Diels-Alder reaction that gives a very different looking product. These two Diels-Alderase enzymes are very similar, so we are now trying to see if and how one evolved from the other through just a few amino acid sequence changes. We hope we can use this as a system to probe how chemical diversity evolved in this plant family, and also to generate enzyme mutants that make non-natural scaffolds from this starting material through other possible Diels Alder reactions.

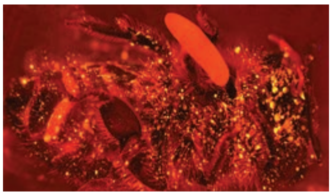
Why did you decide to come to our institute in Jena?

I came for several reasons. First, joining the Max Planck Society ensures that I can think about long-term research projects that take a long period of time. The prospect of being able to do long-term research is very unique. Second, I wanted to start working more on the biology and function of natural products, and MPI-CE is the best institute in the world to do this. Finally, the resources: we now have access to the best tools and equipment to make anything possible. I look at our new mass specs every morning and think about all of the great science that they will enable. Doing great science! This is what I am looking forward to most.

You are the first female director at our institute. What is your advice for young female researchers?

There are two things that come to mind, though these are not necessarily female specific! First, grow a thick skin and don't waste your time taking things personally. Failure and frustration are a big part of science and you have to figure out a way to rebound from them quickly. Try to learn from your failures without getting demoralized. And if someone acts patronizingly towards you (this used to happen to me frequently at the start of my independent research career), just ignore it and worry about more interesting things. Second, find supportive people, both in your professional and in your personal life. At work, you need supporters to help open doors in science and give you advice when you need it. At home, you need supportive people who are okay with the fact that you spend so much of your time and energy doing science. [AO]

Devoted to studying natural products in plants: Sarah O'Connor shows one of her research plants, a Madagascar periwinkle (*Catharanthus roseus*, see also below). She is fascinated by seeing how nature "does" organic chemistry and wants to understand the logic behind how plants assemble the chemical pathways that make these complex molecules. Why does a plant make a natural product in a particular way?
Photos: Sebastian Reuter



A beewolf egg on a dead bee: The release of nitric oxide is made visible as bright spots by the use of a fluorescent dye. *Photo: Erhard Strohm*

Beewolves use a gas to preserve their food

Scientists from the universities of Regensburg and Mainz, and the Max Planck Institute for Chemical Ecology, discovered that the eggs of the European beewolf produce nitric oxide. The gas prevents the larvae's food, dead bees, from getting moldy in the warmth and humidity of the brood cells. The use of this gas to control mold on food supplies has considerably improved the survival of beewolf offspring and represents a key evolutionary invention. [Uni Regensburg]

Original Publication: Strohm, E., Herzner, G., Ruther, J., Kaltenpoth, M., Engl, T. (2019). Nitric oxide radicals are emitted by wasp eggs to kill mold fungi, **eLife** 2019;8:e43718

A receptor protein that is involved in the detection of certain compounds in the feces of caterpillars ensures that the female insects avoid attacked plants. *Photo: Danny Kessler, MPI-CE*

Ovipositing hawkmoths avoid the smell of caterpillar feces

Neuroethology demonstrated that the best oviposition site may be determined not only by plant odors but also by the frass of other larvae of the same species. After determining the repelling substance in the feces of tobacco hornworm larvae which signals the presence of competing conspecifics to the female moths, the researchers identified an odorant receptor which is involved in detecting the typical smell of larval frass; this receptor is thought to govern competition avoidance during oviposition. [AO]

Original Publication: Zhang, J., et al. (2019). The olfactory co-receptor IR8a governs larval feces-mediated competition avoidance in a hawkmoth. **PNAS** 116 (43) 21828-21833

A reunion at the Harnack House of the MPG in Berlin: (from left to right) MPI-CE alumni Amol Fatangare, Ilka Vosteen, Mayuri Napgoda and an unidentified participant. *Photo: private*

Staying in touch with Max Planck

Have you ever wondered about the possibilities of staying connected to the Max Planck Society after leaving an MPI? As a member of the Max Planck Alumni Association (MPAA) and an attendee of the alumni symposium for the past three years, I would like to share my experiences. The two-day career-oriented symposium provides a networking platform for the participants with inspirational talks, panel discussions, and interactive workshops. There are also plenty of social activities to keep you busy. The festive closing session is usually graced with the music of an orchestra and followed by an international dinner. In addition, the alumni and young researchers dress up with colorful costumes unique to their country of origin. This also reflects that the Max Planck family is not confined to Germany, but has spread all over the world. The symposium creates an international atmosphere where bright minds with innovative ideas exchange their thoughts, experiences, and future avenues. I highly recommend joining the MPAA and attend the alumni symposia in the coming years. Mayuri Napagoda

https://www.mpg-alumni.de/

Impressum: PULS/CE is published semi-annually and can be downloaded free of charge on the homepage of the MPI for Chemical Ecology and is distributed electronically as PDF to subscribers. A print version will be sent on request.
Editor: MPI-CE, Jena • Managing Director: Prof. Dr. David G. Heckel (viSdP). Editorial Staff: Dr. Karin Groten, Research Coordination • Angela Overmeyer, M.A., Information and Communication • Emily Wheeler, Editing
ISSN: 2191-7507 (Print), 2191-7639 (Online)