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NEWSLETTER / NOVEMBER 2023

Tracking down the formation of cardenolides

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MAX PLANCK
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EDITORIAL

Going far together

Participants at the Max Planck Partner Group Workshop, "Molecular and Chemical Ecology of Plant Biotic Interactions," in front of the IISER guest house in Pune. In his opening speech, our director Jonathan Gershenzon highlighted the success of our Indian alumnae and alumni, who are now leading their own groups at renowned Indian research institutes and successfully continuing their research in chemical ecology: Samay Pande (Indian Institute of Science), Kesavan Subaharan (Indian Council of Agricultural Research), Sirsha Mitra (Savitribai Phule Pune University), Naveen Bisht (National Institute of Plant Genome Research), Shantanu Shukla (Indian Institute of Science), workshop organizer Sagar Pandit, and Radhika Venkatesan (IISER Kolkata) and Jyothilakshmi Vadassery (National Institute of Plant Genome Research). Five of these scientists are or were leaders of a partner group.
Photo: Angela Overmeyer

Dear readers,

It may be an African proverb, but looking back on the two weeks I was privileged to spend in India in July 2023, I often find myself thinking about it:

> *"If you want to go fast, go alone.
> If you want to go far, go together."*

What better way to describe the need for international research collaboration in the face of global problems?

From July 19 to 23, scientists from Germany and India convened at the Max Planck Partner Group Workshop "Molecular and Chemical Ecology of Plant Biotic Interactions" in Pune, India, organized by Sagar Pandit who is the head of our Max Planck Partner Group at the Indian Institute of Science Education and Research (IISER) (more on page 6). The workshop marked the conclusion of the group's five-year support by the Max Planck Society to study the molecular and chemical ecology of multitrophic interactions between plants, herbivores and their natural enemies.

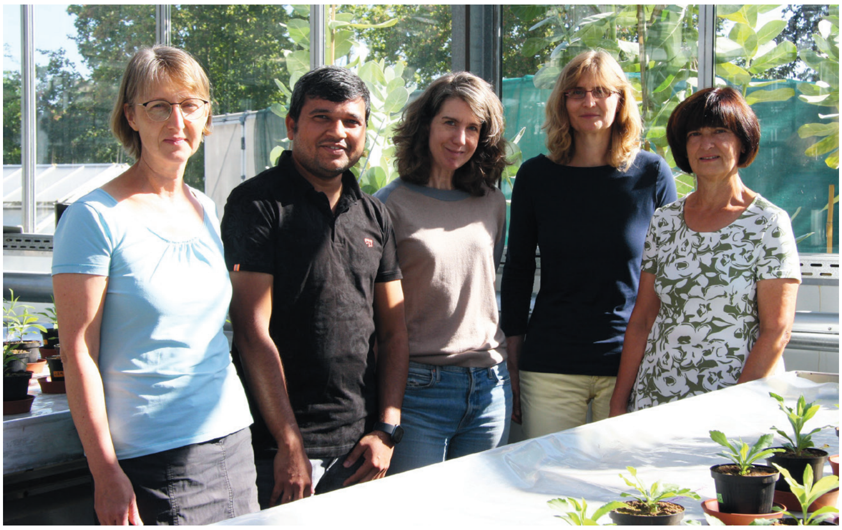
Poonam Sehgal Suri, who represents the Max Planck Society at its India Office in Delhi, also attended the workshop and pointed out that 27 percent of the world's researchers are expected to come from India by 2030. Scientists from India already make up the largest international group of researchers at our institute.

The Partner Group program is an instrument developed by the Max Planck Society to support Max Planck alumnae and alumni in their home countries. Our institute has had fourteen partner groups so far, all of which have received funding from the MPG for up to five years. In addition to six partner groups in India and four in China, we have had one partner group each in Chile, Peru, Kenya and South Africa. As a new collaboration with IISER institutes in India, the Max Planck Society has now launched an IISER-MPG master's internship program to establish early contact with young Indian scientists and to enable Indian master's students to carry out research at Max Planck institutes.

Global issues such as coping with climate change, feeding a growing population, preserving biodiversity while protecting crops and controlling pests clearly show that international cooperation to solve all these problems is more important than ever.

Angela Overmeyer

Tracking down the formation of cardenolides



Plants produce an impressive array of metabolites, including many medically valuable steroids, such as cardiac glycosides, also known as cardenolides. In addition to their effect on the contractility of the heart, cardenolides are also successfully used to treat various cancers. However, until now, the corresponding plant biosynthetic pathways have been largely unknown.

A team of researchers from the Department of Natural Product Biosynthesis is deciphering how plants produce these complex molecules from simple predicted precursors. In addition to the red foxglove *Digitalis purpurea*, which is known to produce cardenolides, the team studied an unrelated plant species, the rubber tree *Calotropis procera*, which also produces large amounts of cardenolides.

The starting point for the study was the suggestion from previous work on a foxglove species that biosynthesis occurs via the molecule pregnenolone. Pregnenolone is also sometimes referred to as the "mother of all steroid hormones,"because in humans all major steroid hormones (testosterone, progesterone and estrogen) can be traced back to this precursor.

The scientists led by Prashant Sonawane identified the candidate genes involved in cardenolide biosynthesis by comparing analyses of both plant species. It was crucial to localize the cardenolides in a tissue-specific manner, so that ultimately only thirteen candidate genes were considered for further characterization. After identifying two enzymes from the cytochrome P450 family 87A that convert both cholesterol and phytosterol into pregnenolone in foxglove and in the rubber tree, the researchers had the first step in the biosynthesis of cardenolides in two distantly related plants. It should be noted that this is the first time that a specific enzymatic function is reported for this subfamily of cytochrome P450 enzymes.

The scientists verified their finding by modifying plants of the model system *Arabidopsis thaliana* in such a way that they produced more CYP87A enzymes. *Arabidopsis* plants genetically modified in this way accumulated unusually high levels of pregnenolone. Further evidence of the involvement of CYP87A enzymes in the formation of pregnenolone was provided by genetically modified foxglove plants that lacked CYP87A enzymes in their leaves. In these plants, the formation of pregnenolone and cardenolides was greatly reduced.

The research team is far from satisfied with deciphering only the first enzymatic step in cardenolide biosynthesis. They want to identify all the downstream steps for the formation of cardenolides in various plant species.

Above: The research team (from left to right): Eva Rothe, who was responsible for the cultivation of the plants; the two lead authors, Prashant Sonawane and Sarah O'Connor; first author Maritta Kunert; and Kerstin Ploss, a tissue culture specialist who helped to establish a stable transformation system for red foxglove. The Department of Natural Product Biosynthesis, led by O'Connor, aims to elucidate the biosynthetic pathways of medically relevant phytochemicals to enable the sustainable production of high-value plant compounds by using other plants as platforms for their biosynthesis.

Middle: The red foxglove Digitalis purpurea *(left), native to Germany, and the rubber tree* Calotropis procera *(right), native to northern Africa.* Photos: Angela Overmeyer

Original Publication: Kunert, M., et al. (2023). A promiscuous CYP87A enzyme activity initiates cardenolide biosynthesis in plants. **Nature Plants**, doi: 10.1038/s41477-023-01515-9

RESEARCH HIGHLIGHT

Protective shield for allies

Above: The white substance that beewolf females secrete from their antennae contains symbiotic bacteria that produce antibiotics to protect beewolf offspring.
Photo: Erhard Strohm

The elongated beewolf egg lies in the brood cell on a captured bee, on which the hatching larva will feed. It releases nitric oxide to kill microbial competitors. On the right the white substance is visible. It protects the symbionts it contains from the toxic gas by using its hydrocarbons as a protective shield.
Photo: Sabrina Köhler

Beewolves, a genus of solitary digger wasps, harbor symbiotic bacteria in their antennae that produce an antibiotic cocktail of up to 49 different substances; this mix protects beewolf larvae efficiently from molds. The females catch honey bees, paralyze the bees with a sting, and drag them into underground brood cells they have dug beforehand. They then also lay their eggs on the bees, which serve as food for the hatching larvae. When laying their eggs, beewolf females deposit the symbiotic bacteria in a white substance on the ceiling of the brood cell.

The beewolf offspring and the bees are constantly threatened by mold fungi, which quickly form in the moist soil of the brood cell and severely limit the storage life of the larvae's food source. Researchers led by Tobias Engl and Martin Kaltenpoth from the Department of Insect Symbiosis knew from previous studies that beewolf eggs release the toxic gas nitric oxide to disinfect the underground brood cell and thus prevent the spread of pathogenic microbes. The research team wanted to know how symbiotic bacteria survive the egg's release of the gas.

To answer this question, they took a closer look at the white substance secreted by the antennal glands of the female beewolf. In particular, the researchers focused on the effect of the secretion and the hydrocarbons it contains against the toxin, nitric oxide. Experiments showed that the whitish secretion from the antennae of the female beewolf, which also contains the symbionts, acts as an effective diffusion barrier. Hydrocarbons contained in it form a protective film that shields the bacterial allies as they are transferred from the beewolf mother to her offspring. The study shows how an insect can protect its own symbionts during the vulnerable phase of transmission from one generation to the next. It also describes another exciting function of hydrocarbons, which in insects primarily serve as protection against desiccation and natural enemies, or play a role in chemical communication.

The beewolf symbiosis is a fascinating case of mutual protection: the symbionts protect the host from pathogens by producing antibiotics. The host, in turn, protects its symbionts from its own defenses against pathogens by producing a layer of hydrocarbons. The mechanism presented here shows how beewolves can defend themselves against pathogens while maintaining symbiotic relationships with their bacterial allies.

Further experiments will clarify whether the special hydrocarbon mixture of the beewolf is particularly well suited to protecting the symbionts or whether any hydrocarbon could perform this task.

Original Publication:
Ingham, C. S. et al. (2023). Host hydrocarbons protect symbiont transmission from a radical host defense. **PNAS**, doi: 10.1073/pnas.2302721120 (2023)

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RESEARCH HIGHLIGHT

Optimimized homing

Desert ants live in the inhospitable salt pans of North Africa. To find food for their nest mates, foraging ants have to walk far into the desert. Once they have found food -- for example, a dead insect -- their real problems begin: how do they find their way back to their nest as quickly as possible in a hot, barren environment?

For years, the research team led by Markus Knaden from the Department of Evolutionary Neuroethology has been studying the incredible ants that find their way back to their own nests unerringly after long treks in barren surroundings in search of food. In previous field experiments in Tunisia, researchers had noticed that nests in the middle of salt pans, where there are few if any visible landmarks, had high mounds at their entrances. In contrast, nest mounds near the shrub-covered edges of the salt pans were low or barely visible. The team therefore wondered if these differences served a purpose and helped the ants find their way home.

For their investigations, the researchers followed the ants using GPS devices. This enabled them to track the animals on their way to the salt pan and back home. The farthest a single animal traveled was more than two kilometers. However, the research team also observed an unexpectedly high mortality rate. About 20% of the foraging ants did not find their way back after extremely long runs and died. Thus, selection pressure to find home ever faster and more target-oriented must be high.

Experiments in which ants could be tracked with particular accuracy during the final meters to the nest, thanks to a grid painted on the ground, demonstrated that nest mounds are important visual orientation cues. If they were removed, not only did fewer ants find their way back to the nest, but their nest mates quickly began to rebuild the nest mounds. If, on the other hand, the scientists placed artificial landmarks in the form of small black cylinders near the nests whose mounds they had previously removed, the ants did not invest in building new mounds. Apparently, the cylinders were sufficient for orientation.

In ant nests, there is a division of labor. Ants that go foraging are usually older and more experienced nest members, while younger ants' tasks include building. Therefore, information must be exchanged between the two groups. Researchers do not yet know exactly how such information transfer is achieved. One possibility is that ants in the nest become aware that the return rate of ants is decreasing, and as a result, efforts to build the nest mound are increased.

Markus Knaden has been studying desert ants for 25 years and is always surprised by their abilities: Despite small brains, these animals are able to process and learn visual and olfactory landmarks. Moreover, they can decide which information is useful for their navigation and which is not. The fact that the desert ants may build their own landmarks for orientation and choose to invest in this work only when other orientation cues are missing has also amazed him.

Desert ants of the species* Cataglyphis fortis *have outstanding navigational skills. With an innate navigation mechanism called "path integration," they use both a solar compass and a step counter to measure the distances they travel. In addition, they can learn and use visible and olfactory cues.
Photo: Markus Knaden

A nest mound helps ants find their way back home after their long foraging trips. However, they build clearly visible nest entrances only when other visual orientation cues are missing.
Photo: Markus Knaden

Original Publication: Freire, M., Bollig, A., Knaden, M. (2023). Absence of visual cues motivates desert ants to build their own landmarks. **Current Biology**, doi: 10.1016/j.cub.2023.05.019

CONFERENCE REPORT

Strong research partners in India

Reuniting with some of our alumni during the International Society of Chemical Ecology meeting in Bangalore.

Below: Sagar Pandit (right) and his wife Sirsha Mitra (center), professor of botany at Savitribai Phule Pune University, with our director Jonathan Gershenzon. Our hosts in Pune are well connected with other Indian alumni of our institute and meet regularly for workshops with their young researchers.

Right: Venkatesh Pal Mahadevan receives the Golden Probe Award.

Photos: Angela Overmeyer

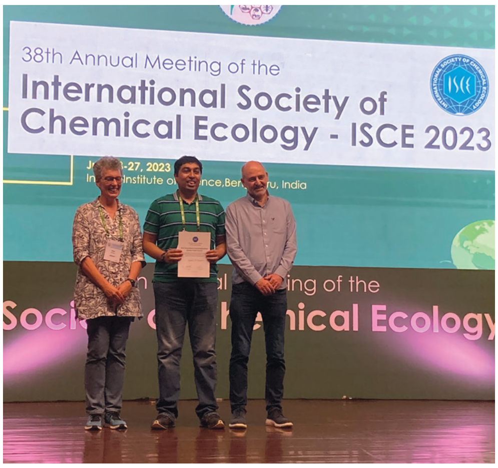
In July, I had the honor to participate as a guest at the Max Planck Partner Group workshop in Pune, together with experts in applied chemical ecology, biotic plant interactions and agricultural entomology in Germany. The workshop was organized by Sagar Pandit, an alumnus of our institute and leader of the partner group. His excellently organized event featured many lectures, poster presentations and cultural program points. and focused on the scientific dialogue between researchers from India and Germany.

Our hosts showed us the impressive research facilities on the IISER campus and demonstrated the high standards with which research is conducted. Grit Kunert, another participant from our institute and head of the Plant-Leaf Aphid Interactions project group, summarized her experience on behalf of the guests from Germany: "What I saw and heard on the IISER campus made a deep impression on me. I was even more impressed by the high level and enthusiasm of the junior scientists. The workshop really made me want to collaborate with Indian partners."

A highlight of the four-day workshop was the excursion to the tropical rainforests of the Western Ghats, one of Asia's most important biodiversity hotspots, where the Indian hosts showed their German colleagues some of the native plants they are studying. One particular image has stuck in my mind: at one point we had to cross a river that had developed a strong current due to the persistent monsoon rains. Everyone had to hold on to each other's hands for safety so as not to be pulled along by the current. Cooperation is important, not only for research!

The subsequent annual meeting of the International Society of Chemical Ecology in Bangalore was another opportunity to reunite with many alumnae and alumni of our institute and to experience more great science as many participants from India presented their research. For our institute, the meeting was a success not only because of the successful exchange. At the end of the meeting, Venkatesh Pal Mahadevan, doctoral researcher in the Department of Evolutionary Neuroethology, was awarded the Student Travel Award and a Poster Award. He also received the Golden Probe Award for the best presentation in electrophysiology. And to cap it off, former director Wilhelm Boland received the Society's Silver Medal for lifetime achievement (page 8).

Angela Overmeyer



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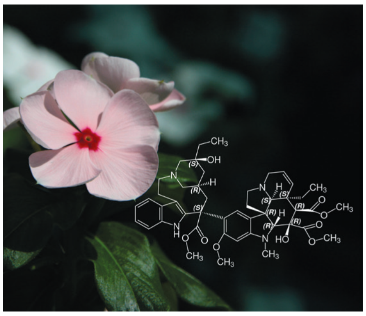
Insights into natural product biosynthesis



The Madagaskar periwinkle (*Catharanthus roseus*) produces the anti-cancer drug vinblastine.
Photo: Angela Overmeyer

An international team of researchers from the University of Georgia, in Athens, USA, and the Department of Natural Product Biosynthesis presented a promising approach to deciphering metabolic pathways that underpin the formation of plant constituents with medicinal significance: single-cell multi-omics reveals the involvement of different cell types in the production and accumulation of medically relevant natural products. The research team studied the biosynthesis of two alkaloids from the plant *Catharanthus roseus* that are used in medicine as anti-cancer agents. The genes for the formation of these active substances are expressed in different cell types. With the help of single-cell analyses, the scientists were able to discover new genes that are important for biosynthesis and to show that the intermediates of the metabolic pathway are enriched in specific cell types. The researchers expect that this methodological approach will provide important new insights into the formation of many other natural products from the plant kingdom.

Original Publication:

Li, C., et al. (2023) Single-cell multi-omics in the medicinal plant *Catharanthus roseus*. **Nature Chemical Biology,** doi: 10.1038/s41589-023-01327-0

The social role determines the risk of infection

Researchers in the Lise Meitner Group Social Behavior, together with international colleagues, have discovered that, given the same genetic makeup, individual behavior alone determines whether or not a member of a social group will contract a pathogen. Clonal raider ants of the species *Ooceraea biroi* that forage outside the nest are more likely to be infected by parasitic nematodes than conspecifics in the nest. The research team also observed that diseases in the colony altered the behavior of all ants: sick and healthy workers alike remained in the nest, and the division of labor was diminished, affecting the overall social organization in the ant colony.

Original Publication:

Li, Z. et al. (2023). Behavioural individuality determines infection risk in clonal ant colonies. **Nature Communications**, 14:5233

Ants are tagged with colored dots for experiments so that the behavior of each individual can be tracked.
Photo: Daniel Kronauer, The Rockefeller University

Genetic diversity is what counts

A team of researchers led by Ian Baldwin showed that natural tobacco mutants impaired in their defenses persist in plant populations by producing more offspring in years of low herbivore pressure. The researchers located and identified the gene that regulates the production of an active defense hormone. Mutants in this gene are susceptible to herbivore attack. However, they compensate for impaired defenses through robust genetic networks. When fewer herbivores are around, mutants grow faster and produce more offspring. Genetic mutations occur frequently. Most often they are harmful, but occasionally they are responsible for traits that facilitate survival in certain environments. In this case, the main mutation may be part of a genetic network and lead to the accumulation of other mutations; the result, increased genetic diversity in natural populations, may help guarantee long-term survival in a changing environment.

Original Publication:

Ray, R. et al. (2023). A persistent major mutation in canonical jasmonate signaling is embedded in an herbivory-elicited gene network. **PNAS** e2308500120

***Coyote tobacco* Nicotiana attenuata.**
Photo: Ian Baldwin

RESEARCH NEWS / AWARDS

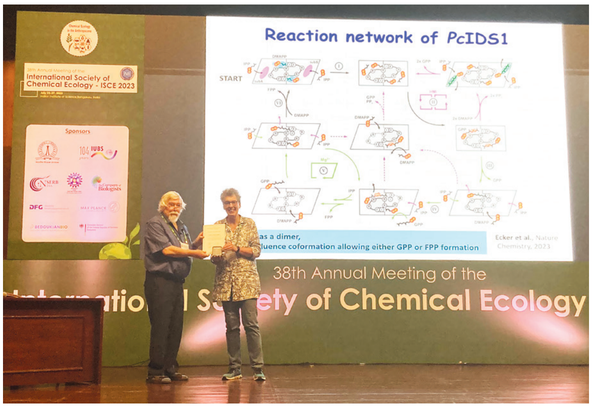
Nature is inventive

Original Publication:

Florean M. et al.(2023). Reinventing metabolic pathways: Independent evolution of benzoxazinoids in flowering plants. **PNAS** doi: 10.1073/pnas.2307981120

Tobias Köllner and Matilde Florean with their research plants. Photo: Angela Overmeyer

Individual species of different plant families produce special indole-derived defense substances, so-called benzoxazinoids. However, the synthetic pathway of these compounds was previously only known for grasses such as maize. By studying two distantly related plant species, the golden dead-nettle *Lamium galeobdolon* and the zebra plant *Aphelandra squarrosa,* researchers from the Department of Natural Product Biosynthesis have been able to show that, compared to maize, completely different enzymes are responsible for the formation of these defense compunds. The biosynthesis of these substances has evolved independently several times during the course of evolution.



Wilhelm Boland receives the Silver Medal from ISCE President Nicole van Dam. Photo: Angela Overmeyer

Wilhelm Boland awarded the Silver Medal of the International Society of Chemical Ecology

Our former director Wilhelm Boland was awarded the Silver Medal of the International Society of Chemical Ecology (ISCE) for outstanding contributions to the field of chemical ecology and for his many years of service to ISCE. In his Silver Medal Lecture, which concluded the 38th ISCE Annual Meeting in Bangalore, India, he presented his research on the topic "Metal matters! Co^{2+} and Mg^{2+} ions control chain length of prenyl diphosphates in insects."

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Martin Kaltenpoth new EMBO member

Martin Kaltenpoth. Photo: Anna Schroll

Martin Kaltenpoth is a new member of the European Molecular Biology Organization (EMBO), the Heidelberg-based scientific organization announced in July. EMBO is a non-profit organization whose aim is to promote research and international exchange in the life sciences. Membership honors distinguished scientists for outstanding contributions to biological research. Kaltenpoth heads the Department of Insect Symbiosis. The evolutionary biologist focuses on symbioses between insects and microorganisms. These partnerships have existed for millions of years. Microbial symbionts play an enormously important role in the ecology and evolution of insects, as they are involved in opening up new habitats, digesting food components and defending against enemies and pathogens. The overarching goal of research conducted by Martin Kaltenpoth and his team is to characterize the diversity and evolution of bacterial symbionts in insects and to investigate their importance for the ecology of the hosts.

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