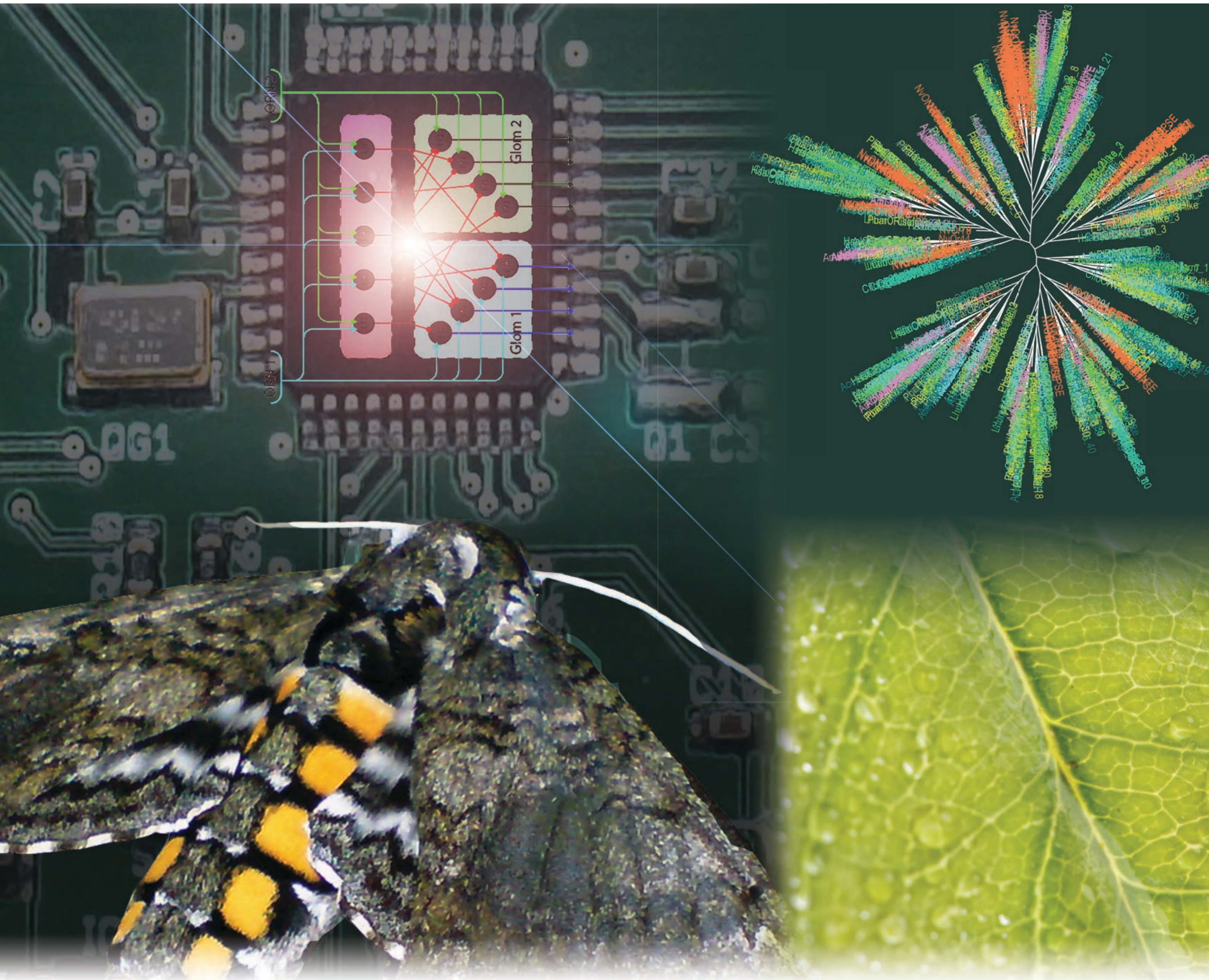
MAX-PLANCK-GESELLSCHAFT

Max Planck Institute
for Chemical Ecology

ICE-12:
THE 12TH INTERNATIONAL COURSE IN CHEMICAL ECOLOGY

FRONTIERS OF
CHEMICAL ECOLOGY



NOVEMBER 26 - DECEMBER 7, 2012
MAX PLANCK INSTITUTE FOR CHEMICAL ECOLOGY
JENA, GERMANY

Special Thanks

To our co-organizers:

Sveriges lantbruksuniversitet

Alnarp, Sweden

Penn State University

University Park, PA, US

To our sponsors:

Volkswagen Foundation

Max Planck Society

Max Planck IMPRS

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The Tobacco Hornworm, *Manduca sexta* in Utah.

ICE-12 FRONTIERS OF CHEMICAL ECOLOGY

Important Information

Location

All course sessions are held at the Max Planck Institute of Chemical Ecology seminar rooms, laboratories, and library

Getting to the Max Planck Institute for Chemical Ecology

- **From the Hotel am Stadion:**
    - Take tram 4, 5, or 35 at tram stop Sportforum to city center
    - At bus stop Teichgraben, Take bus 10, 11, 12, or 13
    - Stop at Beutenberg Campus
    - Walk to first right and proceed up the road to the top of the hill
    - The Institute is the large blue building at the top of the hill, Hans-Knoell Str. 8
- **From the town center:** Follow directions from bus stop Teichgraben above

Accommodation


Hotel am Stadion

All students are housed at the Hotel am Stadion:

Thüringer Sozialakademie gGmbH
Am Stadion 1, 07749 Jena
Tel: 03641 3030
Fax: 03641 303100
Email: info@sozialakademie.info

How to get to the hotel (Hotel am Stadion):

From Jena West train station:

- Take bus line No. 15 in front of the station (Rautal) to the city centre bus terminal located along Teichgraben (or walk for approx. 2 minutes down Westbahnhofstraße and turn right to the bus stop Westbahnhofstraße, where you can take bus line No. 10, 11,12, or 13 (Stadtzentrum) to the city centre. In the city centre (Holzmarkt), take tram line No. 5 or 35 (Lobeda-Ost) to the stop “Sportforum”. Cross the road (Stadtrodaer Straße) and walk approximately 100 m to the left.

From Jena Paradies train station:

- Take the exit to the city (Ausgang Stadt). There is a tram station on the right (tram stop: Paradiesbahnhof), where you can take a tram No. 1, 4, 5 or 35 (Lobeda-West or Lobeda-Ost). Exit at “Sportforum” (two stops). Cross the road (Stadtrodaer Straße) and walk approximately 100 m to the left.

From the Autobahn A4:

- Exit at “Jena-Zentrum” (54) and head towards Jena city centre (Zentrum/Stadion) on Stadtrodaer Straße. Turn right at the traffic lights opposite the sports stadium (Stadion). Turn into the parking lot on the right. Hotel entrance is across the parking lot.

Dining

Weekdays (Monday - Friday afternoon)

All participants will be provided with lunch Monday-Friday at the Beutenberg Campus Mensa (cafeteria). Please have your lunch tickets ready to present to the cashier.

Students with accommodation are provided a breakfast (6:30 - 10:00) and dinner (18:00-22:00) buffet in the hotel dining room.

On Tuesday November 27, a dinner reception will be held directly after the poster session in the institute cafeteria.

Weekend (Friday evening - Sunday)

- **Breakfast:** Available at the Hotel dining room (see Weekdays, above)
- **Lunch:** on Saturday, December 1, a pizza lunch is provided with the literature discussion. On Sunday, December 2, students have free time. Lunch can also be obtained for those traveling to the Dornburg Castles at the cafe.
- **Dinner**: Participants are free to dine on their own. Jena has many restaurants in and around the town center. Please contact one of the MPICE staff for recommendations.

Poster Session

General Information

- **Location:** Max Planck Institute for Chemical Ecology Foyer
- **Date:** November 27, 2012
- **Time**: 14:00-17:00

Reception

A reception will be held immediately following the poster session at 17:00 - 20:00.

Poster Size and Setup

Posters should be approximately 90 X 100 cm in size. Please setup your poster in the institute foyer before 12:00 on November 27 according to registration number.

Contact Information

Course Info

Shannon Olsson: +49 3641 57 1455 (office)
+49 151 43 13 9425 (mobile)
solsson@ice.mpg.de

Payment Info

Reiner Witte: witte@ice.mpg.de

Antje Guedter: aguedter@ice.mpg.de

Emergency

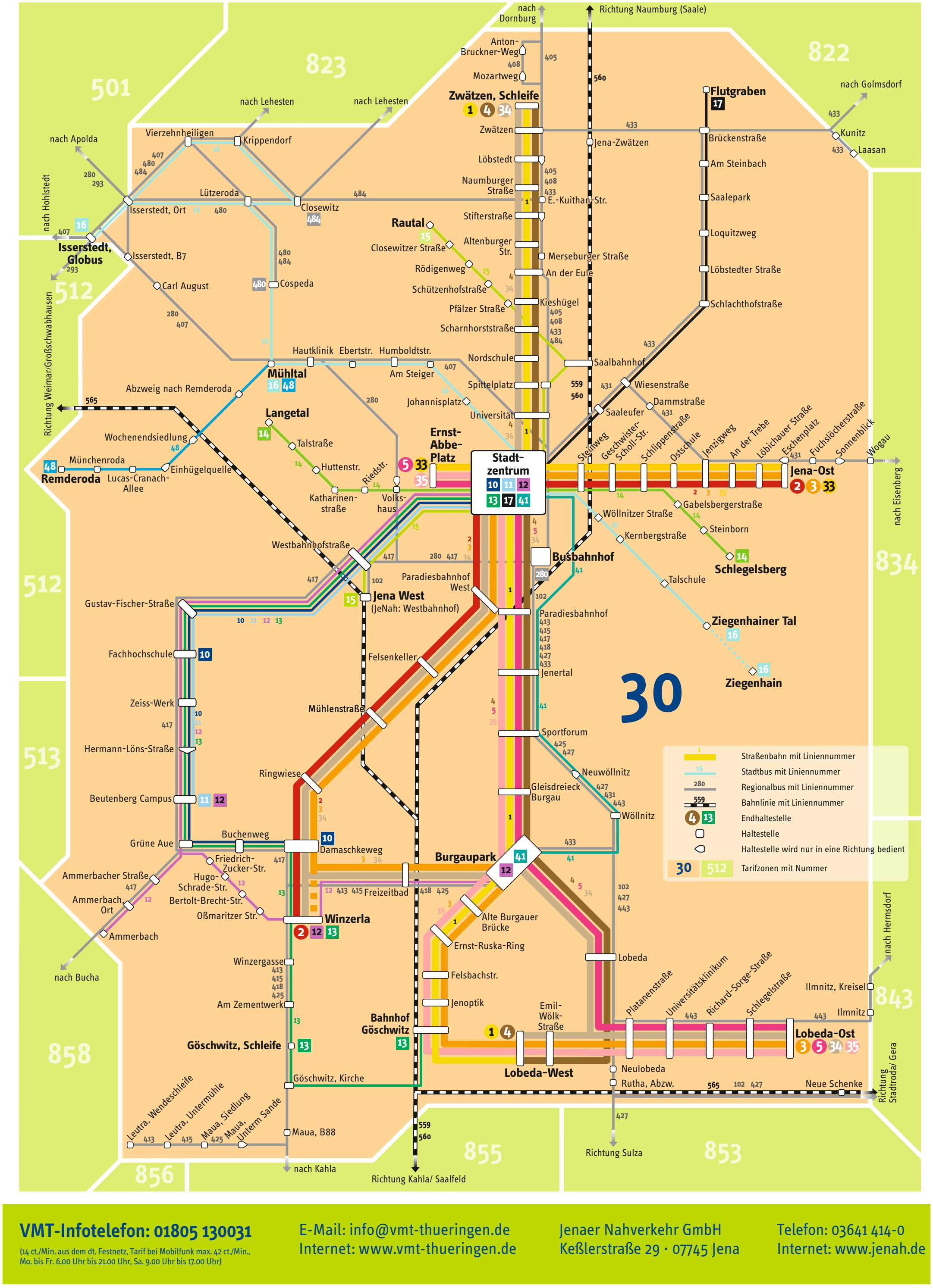
Police department: 110 or 03641/810

Fire department: 112

Accident, life-threatening circumstances, fire, disaster: 112

The ant, *Cataglyphis noda* at a nest entrance in Tunisia.
Copyright: Elisa Badeke, MPI chem. Ökol

Transit Map



501
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Richtung Naumburg (Saale)
nach Dornburg
Anton-Bruckner-Weg
Mozartweg
Zwätzen, Schleife
Zwätzen
Löbstedt
Naumburger Straße
E.-Kuithan-Str.
Stifterstraße
Altenburger Str.
Merseburger Straße
An der Eule
Rautal
Closewitzer Straße
Rödigenweg
Schützenhofstraße
Pfälzer Straße
Scharnhorststraße
Kieshügel
Nordschule
Saalbahnhof
Spittelplatz
Universität
Stadtzentrum
Ernst-Abbe-Platz
Flutgraben
Brückenstraße
Am Steinbach
Saalepark
Loquitzweg
Löbstedter Straße
Schlachthofstraße
nach Golmsdorf
Kunitz
Laasan
Jena-Zwätzen
Wiesenstraße
Dammstraße
Saaleufer
Jena-Ost
nach Eisenberg
Wogau
Wöllnitzer Straße
Gabelsbergerstraße
Kernbergstraße
Steinborn
Schlegelsberg
Talschule
Ziegenhainer Tal
Ziegenhain
Busbahnhof
Paradiesbahnhof
Jenertal
Sportforum
Neuwöllnitz
Gleisdreieck Burgau
Wöllnitz
Burgaupark
Alte Burgauer Brücke
Ernst-Ruska-Ring
Felsbachstr.
Jenoptik
Emil-Wölk-Straße
Lobeda-West
Lobeda
Lobeda-Ost
Neulobeda
Rutha, Abzw.
Neue Schenke
Ilmnitz, Kreisel
Ilmnitz
nach Hermsdorf
Richtung Stadtroda/ Gera
Richtung Sulza
Richtung Kahla/ Saalfeld
nach Kahla
Maua, B88
Göschwitz, Kirche
Göschwitz, Schleife
Bahnhof Göschwitz
Am Zementwerk
Winzergasse
Winzerla
Damaschkeweg
Freizeitbad
Buchenweg
Ringwiese
Mühlenstraße
Felsenkeller
Jena West
(JeNah: Westbahnhof)
Paradiesbahnhof West
Westbahnhofstraße
Katharinenstraße
Volkshaus
Hüttenstr.
Talstraße
Langetal
Mühltal
Hautklinik
Ebertstr.
Humboldtstr.
Am Steiger
Johannisplatz
Abzweig nach Remderoda
Wochenendsiedlung
Münchenroda
Remderoda
Lucas-Cranach-Allee
Einhügelquelle
Richtung Weimar/Großschwabhausen
Isserstedt, Globus
Isserstedt, B7
Isserstedt, Ort
Carl August
Cospeda
Closewitz
Lützeroda
Vierzehnheiligen
Krippendorf
nach Lehesten
nach Apolda
nach Hohlstedt
Gustav-Fischer-Straße
Fachhochschule
Zeiss-Werk
Hermann-Löns-Straße
Beutenberg Campus
Grüne Aue
Ammerbacher Straße
Ammerbach Ort
Ammerbach
nach Bucha
Friedrich-Zucker-Str.
Hugo-Schrade-Str.
Bertolt-Brecht-Str.
Oßmaritzer Str.
Straßenbahn mit Liniennummer
Stadtbus mit Liniennummer
Regionalbus mit Liniennummer
Bahnlinie mit Liniennummer
Endhaltestelle
Haltestelle
Haltestelle wird nur in eine Richtung bedient
Tarifzonen mit Nummer
VMT-Infotelefon: 01805 130031
(14 ct./Min. aus dem dt. Festnetz, Tarif bei Mobilfunk max. 42 ct./Min., Mo. bis Fr. 6.00 Uhr bis 21.00 Uhr, Sa. 9.00 Uhr bis 17.00 Uhr)
E-Mail: info@vmt-thueringen.de
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Keßlerstraße 29 · 07745 Jena
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ICE-12 FRONTIERS OF CHEMICAL ECOLOGY

Program at a Glance

WEEK 1	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
Date	26-Nov	27-Nov	28-Nov	29-Nov	30-Nov	1-Dec	2-Dec
9:00-10:00	Arrival	Institute Tour	PRACTICAL 1 (Ales Svatos: MS; Bernd Schneider: NMR; Tamara Krugel: Greenhouse)	PRACTICAL 1 (Ales Svatos: MS; Bernd Schneider: NMR; Tamara Krugel: Greenhouse)	PRACTICAL 1 (Ales Svatos: MS; Bernd Schneider: NMR; Tamara Krugel: Greenhouse)	Marcus Stensmyr	
10:00-11:00	Introduction/ Orientation						Excursion to Dornburg Castles
11:00-12:00	Bill Hansson	Wilhelm Boland	PRACTICAL 2 (Andreas Reinecke: Insect Phys: Markus Knaden: Behavior; Ewald Grosse-Wilde and Shuqing Xu: Bioinformatics)	PRACTICAL 2 (Andreas Reinecke: Insect Phys: Markus Knaden: Behavior; Ewald Grosse-Wilde and Shuqing Xu: Bioinformatics)	PRACTICAL 2 (Andreas Reinecke: Insect Phys: Markus Knaden: Behavior; Ewald Grosse-Wilde and Shuqing Xu: Bioinformatics)	Markus Knaden	
12:00-14:00	LUNCH	LUNCH	LUNCH	LUNCH	LUNCH	LUNCH	
14:00-15:00	John Pickett		David Heckel	Matthias Erb	Christian Kost		
15:00-16:00		Student Poster Session / Reception				Round Table Discussion / Literature review	
16:00-17:00	Zeyaur Khan		Christer Lofstedt	PUBLISH OR PERISH (John Romeo)	PUBLISH OR PERISH (John Romeo)		
17:00-19:00	STAMMTISCH		STAMMTISCH	STAMMTISCH	STAMMTISCH		

WEEK 2	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY		
Date	3-Dec	4-Dec	5-Dec	6-Dec	7-Dec		
9:00-10:00	Dieter Wicher	Juergen Rybak	Jonathan Gershenzon	Shannon Olsson	Ian Baldwin		
10:00-11:00						(purple)	Special Session
11:00-12:00	Teun Dekker	PRACTICAL 3: Juergen Rybak	Sybille Unsicker	Ring Carde	FAREWELL/ EVALUATION	(green)	Lecture
12:00-14:00	LUNCH	LUNCH	LUNCH	LUNCH		(yellow)	Practical
14:00-15:00	Tom Baker	Peter Witzgall		Silke Sachse		(blue)	Excursion
15:00-16:00			Excursion to Weihnachtsmarkt		Stammtisch/ Departure		
16:00-17:00	Martin Kaltenpoth	Rickard Ignell		Ryohei Kanzaki			
17:00-19:00	STAMMTISCH	STAMMTISCH		STAMMTISCH			

Monday November 26, 2012

10:00 - 10:30: Introduction and Welcome

Shannon Olsson and Bill Hansson
Dept. of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology

10:30 - 10:45 Coffee

10:45 - 12:15: Insect Olfaction: Present state and future directions

Bill Hansson

Most interactions in insect chemical ecology require detection and recognition of volatile chemical signals, be it insect produced pheromones or plant produced kairomones. For such processes to occur on the insect side, an olfactory system is needed. Our present understanding of the insect olfactory system stem from classic investigations in a number of laboratories over the world, stretching from Schneider et al's work in the 1950's to contemporary investigations. To be able to dissect chemical interactions, it is important to have this knowledge up-to-date, but also to look forward at where we need to direct future studies. In my lecture, I will revisit the insect olfactory system and supply some of the basic data that sometimes is forgotten in the molecular era. I will also draw some trajectories from the present state-of-the-art into the future, and make some suggestions regarding phenomena that need to be elucidated.

12:15 - 14:00 Lunch

14:00 - 15:30: New science and practical developments from chemical ecology

John A. Pickett
Dept. of Biological Chemistry, Rothamsted Research

This will comprise an introduction to the potential of secondary metabolism for generating new pest management approaches, and the tools available for its exploitation, from companion cropping to genetically modified crops, will be outlined. Specific opportunities will be discussed involving the disruption of host plant location by interference with host recognition and at the same time potentiating conservation biological control using an understanding of the various chemical ecologically based mechanisms employed by phytophagous insects and their predators and parasitoids. New work will be deptscribed on reducing host acceptance of wheat to pest aphids and increasing foraging by aphid parasitoids by genetic modification so as to produce (*E*)-β-farnesene, the aphid alarm pheromone. Evidence for the success of the approach as determined in the laboratory will be presented and the early development of the first field trial will be shown. This is the first field trial of the underlying ecology and ways further to exploit new understandings of this aspect will be offered, including “switching” on the genes using plant-derived defence elicitors such as cis-jasmone. Finally in this presentation, other opportunities for using such genetic “switching”, particularly for the generation of defence homoterpenes, will be discussed.

Outreach: Outreach is an important issue for the prospect of developing genetic modification (GM) as a tool for delivery of chemical ecologically based pest management. The field trial of the GM wheat in 2012 went ahead because of an extensive process of engagement with the public and has helped to create a “sustaining mutually beneficial and sustainable authentic appropriate relationship”, which is a quoted principle of outreach. Excerpts from the public media will be presented which demonstrate the approaches that we adopted.

Group activity: In groups, after electing a rapporteur and discussion leader, a novel idea for a GM based solution will be developed together with ideas for facilitating an outreach process. These ideas and specific opportunities for outreach activities will then be described to the entire course membership.

15:30 - 17:00: Exploiting chemical ecology in crop protection for smallholder farmers in Africa

Zeyaur Khan
International Centre of Insect Physiology and Ecology (icipe)

Development of a novel push-pull approach for integrated pest and weed management is described. Appropriate plants were discovered that naturally emit signaling chemicals (semiochemicals) that influence insect-plant interactions. Plants highly attractive for egg laying by stemborer pests were selected and employed as trap crops (pull), to draw pests away from the main crop. Of these, Napier grass, *Pennisetum purpureum* (Schumach), despite its attractiveness, supported minimal survival of the pests’ immature stages. Plants that repelled stemborer pests, notably molasses grass, *Melinis minutiflora* P. Beauv., and forage legumes in the genus *Desmodium*, were selected as intercrops (push), which also attracted natural enemies of the pests. *Desmodium* intercrops suppressed parasitic weed, *Striga hermonthica* (Del.) Benth., through an allelopathic mechanism. *Desmodium* root exudates contain novel flavonoid compounds, which stimulate suicidal germination of *S. hermonthica* seeds and dramatically inhibit its attachment to host roots. The companion crops provide valuable forage for farm animals while the leguminous intercrops also improve soil fertility and moisture retention. The system is appropriate as it is based on locally available plants, not expensive external inputs, and fits well with traditional mixed cropping systems in Africa. To date it has been adopted by about 55,000 smallholder farmers in East Africa where maize yields have increased from ~1 t ha^{-1} to 3.5 t ha^{-1}. Opportunities for semiochemical delivery by companion plants, including plant-plant signaling and early herbivory alert, are being explored for developing future smart IPM strategies for Africa.

17:00 - 19:00: Stammtisch

ICE-12 FRONTIERS OF CHEMICAL ECOLOGY

Tuesday November 27, 2012

9:30 - 10:30: The Max Planck Society and its Max Planck Institute for Chemical Ecology in Jena

Jan Kellman
Max Planck Institute for Chemical Ecology

I will give a short intro to the MPG itself, its inception 101 years ago as the "Kaiser Wilhelm Gesellschaft", and then give an overview of some core data about our institute and selected research projects.

10:30 - 10:45 Coffee

10:45 - 12:15: Sequestration of plant-derived glycosides by leaf beetles: a Model system for evolution and adaptation of chemical defenses

Willhelm Boland
Dept. of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology

Leaf beetles larvae have developed an impressive diversity of toxins to defend themselves against predators, which they can discharge from specialized pair wise glandular reservoirs on their back upon attack. The reservoirs represent “bioreactors” performing all late reactions of the toxin-production, starting from plant-derived (sequestered) or *de novo* synthesized glucosides of non-toxic, early precursors of their genuine defenses.

The initial uptake of plant-derived glucosides by the larvae’s intestine seems to be fairly unspecific, which contrasts sharply with the specific import of precursors into the defensive glands. The Malpighian tubules and hind-gut organs facilitate the rapid clearing of body fluid from excess or unusable compounds. Thus, an interconnected network of export and import of glycosides exists in both sequestering species and species producing early precursors of the deterrents *de novo* in their fat body. The successful combination of the pathways of excretion and defense has probably allowed them to adaptively radiate onto, and co-evolve with plants that offer appropriate glycoside precursors. Such a dual system of de novo biosynthesis and sequestration of phytogenic precursors for the production of defenses may have allowed the larvae to shift from one host plant to another without losing their defense albeit sometimes with enhanced costs of production.

Systematic cloning and expression of the first enzymes of the biosynthetic machineries producing iridoids *de novo* or salicylaldehyde after sequestration of the phenolic glucoside salicin into the "bioreactors" of the defensive systems, gave first insights into the mode of regulation (sequestration versus *de novo* biosynthesis), as well as into the genetic modification of biosynthetic genes and enzymes after a host plant shift caused by excessive parasitism of the leaf beetle larvae.

M. Kunert, A. Søe, S. Bartram, J. Pasteels, S. Discher, K. Tolzin-Banasch, L. Nie, A. David & W. Boland. *De novo* biosynthesis versus sequestration: a network of transport systems supports in ancestral leaf beetle larvae both modes for the production of iridoid defenses. (2008) Insect Biochem. & Mol. Biol., 38, 895–904.

S. Discher, A. Burse, K. Tolzin-Banasch, S. Heinemann, J.M. Pasteels & W. Boland. Chrysomelina larvae possess a transport network for sequestering and excreting plant glucosides as a source for chemical defense. (2009), ChemBioChem, 10, 2223 – 2229.

R. Kirsch, H. Vogel, A. Muck, J.M. Pasteels & W. Boland. Host plant shifts affect a major defense enzyme in *Chrysomela lapponica*. (2011) Proc. Natl. Acad. Sci., USA., 108, 4897-4901.

R. Bodemann, A Burse & W. Boland. Precise RNAi-mediated silencing of metabolically active proteins in the defence secretions of juvenile leaf beetles RNAi in leaf beetles. (2012) Proc. Royal Soc. Ser. B., 279, 4126–4134.

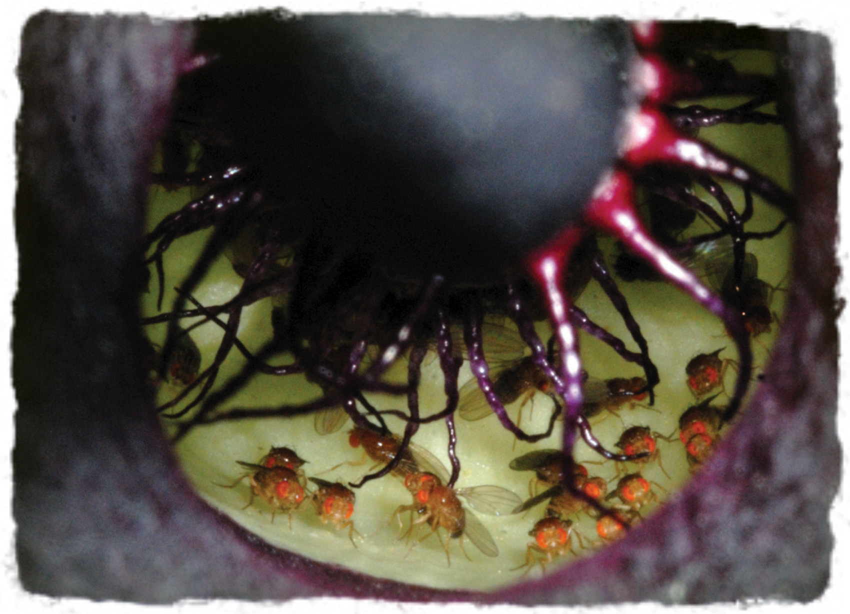
12:15 - 14:00 Lunch

14:00 - 17:00: ICE-12 Student Poster Session

Institute Foyer

17:00 - 20:00: Reception

Institute Cafeteria



Fruit flies trapped in an *Arum palestinium* flower
Copyright:, Stökl, MPI chem. Ökol.

Wednesday November 28, 2012

9:00 - 10:30: Practicals 1

Session 1: Aleš Svatoš

Research Group Mass Spectrometry/Proteomics, Max Planck Institute for Chemical Ecology

Mass spectrometry (MS) is a leading method for analysis of organic compounds due to high sensitivity and versatility. One of the MS, method gas chromatography – MS (GC-MS), has been successfully used for analysis of semiochemicals (plant volatiles, sex pheromones etc.). Now, non-volatile molecules can be analyzed as well and MS goes beyond classical GC-MS scenario, and allows for detection and identification of secondary metabolites or proteins. MS linked with a separation method like liquid chromatography in ultra-performance (UPLC) or nano formats (nonoUPLC) is a working horse for metabolomics and proteomics chemo-ecological studies. In this session a short introductory seminar will summarize MS methods potentially useful in chemical ecology and will give participants a primer for MS fundamentals and technology. In the practical part four different applications will be shortly presented. There will include high-resolution GC-MS, metabolomics, proteomics and MALDI-MS imaging.

Ullmann-Zeunert, L., Muck, A., Wielsch, N., Hufsky, F., Stanton, M., Bartram, S., Böcker, S., Baldwin, I. T., Groten, K., Svatoš, A. (2012). Determination of ^{15}N-incorporation into plant proteins and their absolute quantitation: a new tool to study nitrogen flux dynamics and protein pool sizes elicited by plant-herbivore interactions. Journal of Proteome Research. doi:10.1021/pr300465n

Svatos, A. (2010). Mass spectrometric imaging of small molecules. Trends in Biotechnology, 28, 425-434.

Moco, S., Schneider, B., Vervoort, J. (2009). Plant micrometabolomics: the analysis of endogenous metabolites present in a plant cell or tissue. Journal of Proteome Research, 8(4), 1694-1703.

Session 2: Bernd Schneider

Research Group Biosynthesis/NMR, Max Planck Institute for Chemical Ecology

NMR spectroscopy is the most informative method for structure elucidation of natural products. Some important features are

- Direct information about connectivities between atoms of a molecule
- Distinguishing isomers
- Useful to analyze compounds of any polarity. No derivatization required
- Non-destructive method - sample can be recovered after analysis
- Quantification of metabolites in mixture

- Isotope labelling (^{13}C, ^{2}H, ^{15}N) for biosynthetic studies
- Optional hyphenation with LC and MS

Drawbacks: - Moderate sensitivity
- Spectrometers are expensive

Applications of NMR in chemical ecology and natural product chemistry:

- Purity check
- Identification of known compounds by ^{1}H NMR or ^{13}C NMR
- *De novo* structure elucidation
- Metabolic profiling and metabolomics studies
- Quantification
- Biosynthesis and metabolization
- Organ-, tissue-, and cell specific localization of natural products.

Technical demonstration in the NMR lab will show:

- Sample preparation
- Adjustments of the spectrometer for data acquisition
- Data acquisition
- Processing of spectra
- Interpretation of spectra

Session 3: Tamara Krügel

Greenhouse, Plant Cultivation, Max Planck Institute for Chemical Ecology

The greenhouse of the Max Planck Institute for Chemical Ecology is a service facility for the whole institute. Research plants are grown here using different culture techniques depending on the scientific questions raised, and experiments are also performed. The whole greenhouse facility is divided into two areas: the glass house and the breeding facilities with fully climatized walk-in chambers and small movable chambers. Both areas have computer-operated climate control. Advantages and problems of climatization in the greenhouse will be discussed. We perform biological pest control in the greenhouse by applying predatory or parasitoid insects and arachnids to control pests. For some years now, we have our own culture of beneficial insects. These cultures of biological control agents will be shown as well as a short film on beneficial insects in action. Our list of plants includes about 500 plant species. Some of them are cultured only for a short time. Others, like model plants *Nicotiana attenuata* and *Arabidopsis thaliana* have been continuously cultivated in large numbers. Many of these plants are genetically modified. An overview of the developmental stages of the most important plant species will be provided. Some problem of safety in genetic engineering facilities associated with genetically modified plants will be discussed.

10:30 - 10:45 Coffee

10:45 - 12:15: Practicals 2

Session 1: Andreas Reinecke:

Dept. of Behavioural Ecology and Evolutionary Genetics, Max Planck Institute for Ornithology

Knowing the nose - electrophysiological recordings from insect antennae: Olfaction is the core sense for most insects when locating a mate or assessing any kind of resource from a distance. Understanding olfaction driven behavior requires knowing the "nose". You will get a practical introduction into two principal techniques: (i) Electroantennographic detection (EAD) - recording from the whole antenna and (ii) single sensillum recordings (SSR) - understanding how individual sensory neurons contribute to deciphering chemical information present in the environment. We will discuss a number of scientific questions from both applied and up to date fundamental perspectives and identify the technique best suited to generate an answer.

Session 2: Markus Knaden

Dept. of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology

During the practicals you will learn how flies and moths can be tested for olfactory preferences. Together with my students I will show and explain the "Flywalk", a paradigm that allows us to investigate the flies' responses in so far unmatched accuracy. You will furthermore see our wind tunnel in action. Within this wind tunnel, we fly sphingid moths and test their preferences for host plants and flower odors.

Session 3: Ewald Große-Wilde and Shuqing Xu

Depts. Evolutionary Neuroethology and Molecular Ecology, Max Planck Institute for Chemical Ecology

Next generation sequencing (NGS) techniques are opening many new opportunities in biology. Among many different NGS techniques, RNA-seq is frequently employed in chemical ecology. In this course, we will introduce the use of RNA-Seq and accompanying bioinformatics in chemical ecological studies, starting with identification of putative gene functions, and functional overview of tissues/species using modern transcriptomics. In the second part we will demonstrate how to analyse gene expression using RNA-seq data on an open, web-based platform (Galaxy); and explain how to apply comparative transcriptome sequencing approach to identify candidate genes that are likely involved in chemical communications between plants and insect. After this course, we hope students will have an overview on how to apply RNA-seq in their own study systems and available bioinformatic tools for RNA-seq data analysis.

12:15 - 14:00 Lunch

14:00 - 15:30: Genetics of sex pheromone systems in Lepidoptera

David Heckel
Dept. of Entomology, Max Planck Institute for Chemical Ecology

In the sexual communication system of moths, females produce a species-specific blend of volatile compounds that attract males over long distances for mating. There is a dilemma in explaining how new species with different pheromone systems can evolve: male and female traits are encoded by different genes, and development of a new system would seem to require simultaneous changes in both. To identify these genes, crossing of closely related species or populations with pheromone differences can be used in a genetic approach. Strategies for construction of high-resolution linkage maps using AFLPs and RAD tags will be outlined, which exploit the unique absence of crossing-over in female Lepidoptera. QTL mapping of male and female phenotypes will be described, and the identification and validation of candidate genes for variation in female pheromone production and male behavior will be illustrated, with special focus on *Heliothis* spp. and *Ostrinia nubilali*s.

15:30 - 17:00: Coevolution of sex pheromone production and response in moths

Christer Löfstedt
Pheromone Group, Dept. of Biology, Lund University

Both species-specific pheromone production and response are controlled by multi gene families. I will examine the influence of genetic variation on production and response including examples of site-directed mutagenesis, discuss selective processes molding the systems, and finally bring up the potential of exploiting this knowledge in the semi-synthetic production of pheromones by the use of cell factories.

17:00 - 19:00: Stammtisch

Thursday November 29, 2012

9:00 - 10:30: Practicals 1

See Wednesday November 28, 2012 for Details

10:30 - 10:45 Coffee

10:45 - 12:15: Practicals 2

See Wednesday November 28, 2012 for Details

12:15 - 14:00 Lunch

14:00 - 15:30: Dark magic? Chemical ecology of root-herbivore interactions

Matthias Erb
Group Leader "Root-Herbivore Interactions", Max Planck Institute for Chemical Ecology

Root herbivores are important ecological drivers and agricultural pests, but only recently have we begun to appreciate the importance of the chemical signals that mediate their behavior and interaction with plant roots. During my lecture, I will cover recent findings on how root herbivores locate plant roots using volatile cues, how they optimize their spatial foraging by sensing plant metabolites and how they cope with root toxins via metabolic and behavioral adaptations. Furthermore, I will highlight how roots respond to insect herbivores and these responses affect other herbivores and higher trophic levels. Special emphasis will be put on how root-herbivore interactions differ from well-known above ground systems. In a set of exercises based on a compare& contrast approach, students will have the possibility of identifying interesting questions in their own field of study.

15:30 - 17:00: Special Workshop: The fourth stage -- Write it well and get it published, Part 1

John Romeo
Editor-in-Chief, Journal of Chemical Ecology; Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida

The 8 criteria required for peer review and possible publication in the Journal of Chemical Ecology will be discussed. Use of electronic media for writing preparation, submission/review, and getting noticed is essential. The importance of a carefully chosen title and well-crafted abstract will be emphasized as a way of bringing interest and attention to your research. We will do some exercises. The rules are not complicated. Suggestions for dealing with: the challenges of writing in English (good grammar isn't enough); structuring papers properly (what goes where); data presentation (tables vs, figures); referencing (when, where, and how much); and how to avoid common causes of confusion, misunderstanding, and rejection (lack of context, redundancy, overstatement) will be noted. Currents concerns of publishers include: LPUs (least publishable units); inadequate scope and novelty of the research; faulty methodology (especially too little chemistry and wrong statistics); plagiarism (words and ideas); and poor English. Any of these can torpedo your papers. Some do's and don'ts will be suggested.

17:00 - 19:00: Stammtisch

The Tobacco Hornworm, *Manduca sexta,* feeding from the nectar of a tobacco flower.
Copyright: Copyright: D. Kessler, MPI chem. Ökol.

Friday November 30, 2012

9:00 - 10:30: Practicals 1

See Wednesday November 28, 2012 for Details

10:30 - 10:45 Coffee

10:45 - 12:15: Practicals 2

See Wednesday November 28, 2012 for Details

12:15 - 14:00 Lunch

14:00 - 15:30: Small but GREAT - The power of using microbes to address fundamental questions in chemical ecology

Christian Kost
Experimental Ecology and Evolution Group, Max Planck Institute for Chemical Ecology

The field of chemical ecology is traditionally focussing on animal and plant systems, thereby overlooking the wealth of exciting research opportunities the bacterial world has to offer. In recent years, scientists have started to also include fungi and bacteria into their perspective, thereby discovering that microbes are often key players that govern multipartite interactions between macroorganisms. Moreover, microbes show a plethora of sophisticated behaviours that remarkably resemble the ones displayed by macroscopic systems including sexual communication, allelopathy, and induced defences. While interesting in their own right, these behaviours can also serve as a model to understand their evolution in general. Since in many cases the genetic and biochemical underpinnings of these interactions are very well understood, they are amenable to genetic manipulation. By performing rigorous laboratory-based experiments with defined mutants over both ecological or evolutionary time-scales, the ultimate evolutionary causes for these behaviours can be identified. I will argue that combining molecular and microbiological methodology with ecological and evolutionary theory provides a valuable tool to address fundamental questions in chemical ecology.

15:30 - 17:00: Special Workshop: The fourth stage -- Write it well and get it published, Part 2

See Thursday, November 29, 2012 for details

17:00 - 19:00: Stammtisch

Maize pests in action: armyworm *Spodoptera frugiperda*.
Copyright: Matthias Erb, MPI chem. Ecol.

Saturday December 1, 2012

9:00 - 10:30: Comparative olfactory neuroethology

Marcus Stensmyr
Dept. of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology

The olfactory system directly interfaces with the environment, thus, changes in the environment, or a change in an animal's habits, as e.g. specialization, would presumably also lead to changes in the olfactory system. Comparative studies on specialized animals, with known generalist ancestors, could hence be a way of revealing general processes shaping olfactory systems, as well as highlighting importance and function of specific chemosensory genes. In this presentation I will outline current work in our laboratory dealing with olfactory adaptations at the molecular, physiological, morphological and behavioral level in a set of highly specialized drosophilid flies.

10:30 - 10:45 Coffee

10:45 - 12:15: Odor-guided behavior in ants and flies

Markus Knaden
Dept. of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology

Insects use odors to pinpoint e.g. food and mating partners.

A. the ant case: So far the desert ant *Cataglyphis fortis* is well known for its visually-guided navigation capabilities. During the past years it became more and more evident that the olfactory sense plays a major role in targeting food items and localizing the visually inconspicuous nest entrance. Here I will present field data that suggest that the ants use a sophisticated strategy to screen their harsh habitat for tiny food items efficiently.

B. the fly case: vinegar flies are well known to target food sources based on olfactory cues. By using the newly established behavioral paradigm "Flywalk", we could show that responses to food odors are sex specific and that the flies are able to use olfaction to estimate the mating status of conspecifics.

12:15 - 14:00 Lunch

14:00 - 16:00: Literature review/Round table discussion: What is the Frontier of Chemical Ecology?

Literature Review

We will first discuss the concepts outlined in the two articles provided (see Articles). Students should read the articles prior to the discussion.

Round Table Discussion

As a group exercise, we will discuss the major upcoming topics in Chemical Ecology and their underlying concepts. Students should come prepared with a few ideas of where they believe the field is or should be heading in the coming years.

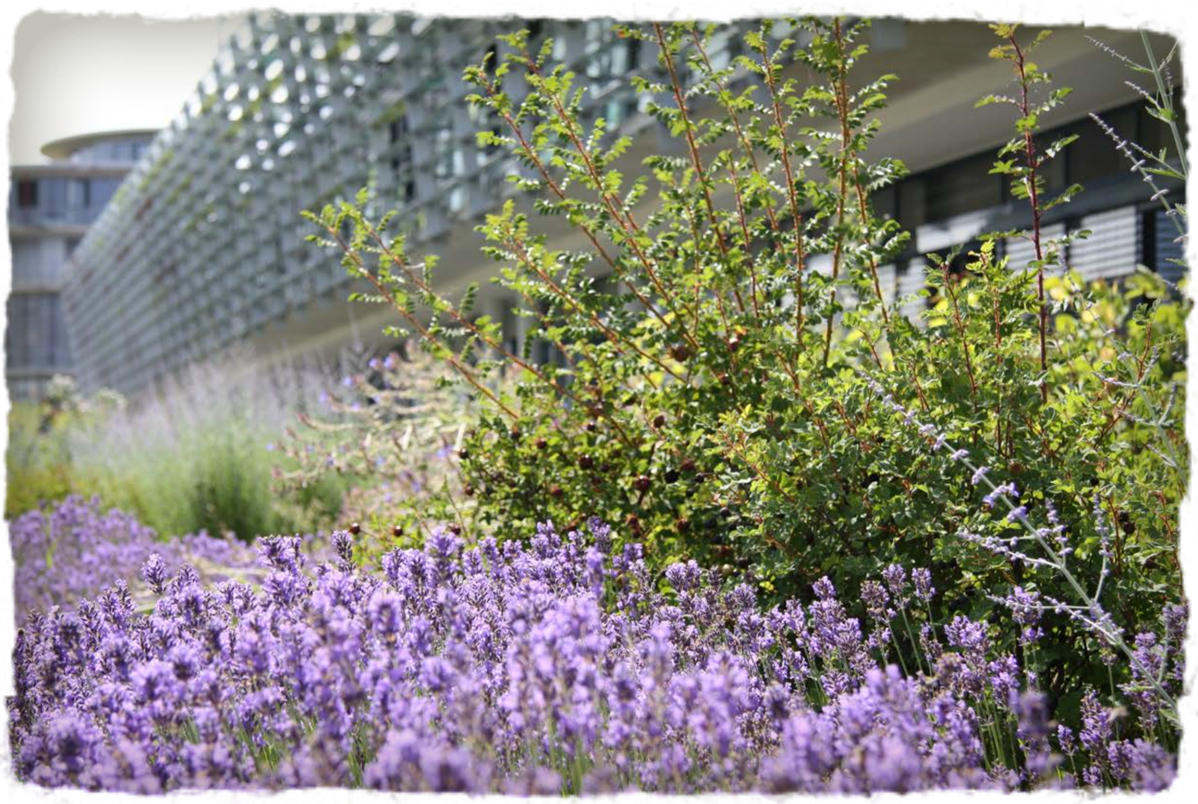
Leafhopper of the genus *Empoasca*.
Copyright: Michael Stitz, MPI chem. Ökol.

16:00 - 18:00: Stammtisch

Sunday December 2, 2012

13:00 - 17:00: Excursion to Dornburg Castles

Bus Departs Hotel am Stadion at 13:00



The Max Planck Institute for Chemical Ecology on a warm sunny day.

Monday December 3, 2012

9:00 - 10:30: Insect odorant receptor function and signaling

Dieter Wicher
Dept. of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology

To perceive and process odor information is of central importance for the interaction of an insect with its environment. In response to odorant binding to respective receptors, a chemical message is transduced into an electrical nerve pulse and further processed in the brain. Insect odorant receptor (OR) proteins have a 7-transmembrane topology like GPCRs. But there is no sequence similarity to other GPCRs and the OR proteins are inversely oriented in the membrane. The insect ORs are heterodimers composed of an odor-specific OR protein and a co-receptor with chaperone function. The primary odor response of these ORs is ionotropic, yet there are indications that odors also initiate metabotropic signaling. The talk presents the current knowledge on insect odorant receptor function and shows the role of metabotropic signaling in regulating the OR sensitivity.

10:30 - 10:45 Coffee

10:45 - 12:15: Evolution of insect olfaction

Teun Dekker
Chemical Ecology, Dept. of Plant Protection Biology, SLU Alnarp

Volatile organic compounds (VOCs) are exquisite information vehicles of resource identity and quality. Whereas we, relatively anosmic humans, may not fully appreciate their potential, most insects have long capitalized on the enormous coding capacity of VOCs by having a large share of their sensing and processing capabilities devoted to olfaction. An array of 50+ different sensory neuron types underlies the detection of VOC, rendering it a sense of unsurpassed multidimensionality. Indeed VOC signals can therefore, in theory, not easily be misinterpreted and constitute genuine 'signatures' of resource identity and quality. Considering the enormous niche diversity, VOCs constitute a primary means for small sized and short-lived insects to innately and efficiently 'navigate' through landscapes. The evolutionary corollary of this is that olfaction is a prime 'target' in niche differentiation and speciation. This will be illustrated in this lecture.

Studies on the evolutionary dynamics of the olfactory circuit are also very helpful for our understanding of how olfactory preference itself is encoded in the circuitry. This is important as, in spite of much progress, understanding how insects make sense of incoming sensory patterns, is still in its infancy. Neural codes that are at the basis of odor-mediated behaviors such as 'attraction' and 'repulsion' are ill described. Even for the most well-studied model, the banana fly, it is largely unclear what makes banana to banana. Studying how niche shifts are accommodated by changes in the olfactory circuit helps us define elements that shape olfactory preference.

Research on evolution of insect olfaction ultimately highlights how insects are able to mold their senses to our agroecosystems. Perhaps more accurately phrased though: the research highlights how we mould our agroecosystems to fit particular species, as the enormous species polymorphic diversity in insect inevitably harbor or will 'produce' species that are fitting the niches we create. By studying how coding features 'evolve', we will get a better grip on how insects utilize chemical cues in a context dependent manner, and consequently, how we, humans, can harness this knowledge in designing novel, intelligent and sustainable ways of controlling pests, through targeting the insect and/or through altering our cropping systems.

12:15 - 14:00 Lunch

14:00 - 15:30: The neuroethology of chemical ecology

Tom Baker
Chemical Ecology Laboratory, Dept. of Entomology, Penn State University

My lecture(s) will focus on the importance of linking behavioral responses important to reproduction, such as attraction (or repellency) to pheromones, food odors, or oviposition-site-related odors, with the neurophysiological responses of olfactory receptor neurons to these odors. A new frontier concerning this type of neuroethological approach to chemical ecology concerns understanding the importance of odor flux, and not odor concentration; it is becoming quite apparent that receptor neurons are endowed with an extremely high, sub-second temporal resolution ability that is reflected in the resulting behavioral responses when discriminating behavioral bioassays are employed.

15:30 - 17:00: The chemical ecology of symbiotic interactions with microorganisms

Martin Kaltenpoth
Insect Symbiosis Research Group, Max Planck Institute for Chemical Ecology

Symbiotic alliances with bacteria play enormously important roles for the ecology and evolution of all eukaryotic organisms. Despite intensive research of the past decades, however, little is yet known about the chemical interactions that mediate host-symbiont recognition and regulate the maintenance of the mutualistic association. After a brief introduction on the importance of eukaryote-bacteria symbioses and the functions that can be conferred by bacterial mutualists, we will explore what is known about the molecular interactions in some model associations and try to come up with general patterns and open questions in this exciting research area.

17:00 - 19:00: Stammtisch

Tuesday December 4, 2012

9:00 - 10:30: Mapping the invertebrate connectome

Jürgen Rybak
Dept. of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology

The reconstruction of neural circuits, underlying chemosensory perception, neural computation and olfactory-driven behavior is studied with a multiple repertoire of neuroanatomy tools. how can we analyze complex neural circuits of an animal by means of neuroanatomy, on the mesoscopic scale, i.e. connectivity of brain regions, to nanoscale dimensions revealing the synaptic communication in local brain circuits? Mapping the connectome takes two complementary approaches: sparse sampling of structural data by pairwise comparison at single cell resolution versus high-throughput analysis as employed in automated electron microscopy or light microscopic-based genetic labeling (Brainbow, GRASP) that provides statistical assumption at the neuron population level. Bioinformatics plays an important role by providing algorithm to integrate structural data into databases (e.g.brain atlases) by use of image registration. Finally, how do we handle and query ontology-based databases that combine functional properties (optical imaging, electrophysiology, molecular) across scales and modalities (system-cellular-subcellular- genes).

10:30 - 10:45 Coffee

10:45 - 12:15: Practical 3: Digital anatomy and bioinformatics

Jürgen Rybak
Dept. of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology

Introduction to basics of image registration, by integration of neural data to standard brain templates and the use of ontology based databases using selected examples (electron microscopy /confocal microscopy) of Drosophila and other insects.

Recommended literature:

- Virtual Fly Brain: http://www.virtualflybrain.org/site/vfb_site/home.htm
- The Honeybee Brain Atlas: http://www.neurobiologie.fu-berlin.de/beebrain/
- Barcoding neurons: Zador AM, Dubnau J, Oyibo HK, Zhan H, Cao G, Peikon ID. 2012.
- Sequencing the Connectome. PLoS Biol 10(10) :e1001411. doi: 10.1371/journal.pbio.1001411 http://www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.1001411?utm_source=feedburner&utm_medium=feed&utm_campaign=Feed%3A+plosbiology%2FNewArticles+%28Ambra+-+Biology+New+Articles%29

12:15 - 14:00 Lunch

14:00 - 15:30: Semiochemicals as a green, sustainable pest management tactic

Peter Witzgall
Chemical Ecology, Dept. of Plant Protection Biology, SLU Alnarp

The idea of using species-specific behavior-modifying chemicals for the management of noxious insects in agriculture, horticulture, forestry, stored products and for insect vectors of diseases, has been a driving ambition through five decades of pheromone research. Hundreds of pheromones and other semiochemicals have been discovered that are used to monitor the presence and abundance of insects and to protect plants and animals against insects. The estimated annual production of lures for monitoring and mass trapping is in the order of tens of millions, covering at least 10 million hectares. Insect populations are controlled by air permeation and attract-and-kill techniques on at least 1 million hectares. We here review the most important and widespread practical applications. Pheromones are increasingly efficient at low population densities, they do not adversely effect natural enemies, and can therefore bring about a long-term reduction in insect populations, which cannot be accomplished with conventional insecticides. A changing climate with higher growing season temperatures and altered rainfall patterns makes control of native and invasive insects an increasingly urgent challenge. Intensified insecticide use will not provide a solution, but pheromones and other semiochemicals can instead be implemented for sustainable areawide management and will thus improve food security for a growing population. Given the scale of the challenges we face to mitigate the impacts of climate change, the time is now right to intensify goal-oriented interdisciplinary research on semiochemicals, involving chemists, entomologists and plant protection experts, in order to provide the urgently needed, sustainable and cost-effective technical solutions for sustainable insect management worldwide.

15:30 - 17:00: Mosquito Olfaction

Rickard Ignell
Chemical Ecology, Dept. of Plant Protection Biology, SLU Alnarp

Mosquitoes that act as disease vectors rely upon olfactory cues to direct several important behaviors that are fundamentally involved in establishing their overall vectorial capacity. In my lecture, I will present recent advances in molecular and physiological investigations into the mechanisms regulating mosquito host preference. Furthermore, I will introduce the origin of vertebrate host blood feeding and the evolution of the host seeking behaviour.

The Yellow Fever mosquito, *Aedes aegypti,* on human skin.

17:00 - 19:00: Stammtisch

Wednesday December 5, 2012

9:00 - 10:30: Prepared for poison: How herbivores circumvent plant defenses

Jonathan Gershenzon
Dept. of Biochemistry, Max Planck Institute for Chemical Ecology

Despite the vast arsenal of apparent chemical defense compounds in most plant species, insect herbivores often eat large amounts of plant tissue without any obvious ill effects. We will explore the mechanisms that herbivores employ to avoid being poisoned by plant defenses. Much attention will be devoted to detoxification processes involving the metabolic transformation of defenses by enzymes such as cytochrome P450s and glutathione-S-transferases. However, we will also consider the roles of excretion and sequestration, and how herbivores can sometimes make the intended molecular target of a defense insensitive to its action. Some of the results discussed will be drawn from the literature on insecticide resistance since there are still many gaps in our knowledge of resistance to natural plant defenses. In addition to the mechanisms themselves, we will also compare the costs and benefits of circumventing plant defenses and how these might explain herbivore host ranges and the evolutionary radiation of herbivore species. In particular, the generalist and specialist feeding strategies will be contrasted. Mention will also be made of the methods used in studying herbivore avoidance of defenses, including the benefits of modern chemical and gene sequencing technologies.

10:30 - 10:45 Coffee

10:45 - 12:15: Plant volatiles in biotic interactions

Sybille Unsicker
Dept. of Biochemistry, Max Planck Institute for Chemical Ecology

When plants are attacked by insect herbivores, they release complex mixtures of volatiles. These compounds can either directly repel the attackers or indirectly protect plants by attracting herbivore enemies, such as parasitic wasps and predatory arthropods. There is now also ample evidence that volatiles are important mediators of intra- and inter-plant communication. So far the role of volatiles in interactions of plants with their biotic environment was mainly studied in agriculturally important crops such as maize, tomato, lima bean and model organisms like *Arabidopsis thaliana* and *Nicotiana attenuata*. The experiments were mostly performed under controlled greenhouse and laboratory conditions and only rarely has plant volatile emission been investigated under natural field conditions. In my seminar, I will cover recent findings of volatile emission from plants as a response to biotic stresses such as insect herbivory and plant competition. I will contrast laboratory and field experiments in herbaceous and woody plant species. Furthermore, spatial aspects of volatile emission and the role of herbivore induced volatiles in indirect plant defense will be addressed.

12:15 - 14:00 Lunch

14:00: Excursion to Weihnachtsmarkt

A projection neuron in the hawkmoth antennal lobe
by Linda Kuebler and Shannon Olsson

Thursday December 6, 2012

9:00 - 10:30: Odor objects in the insect world

Shannon Olsson
Dept. of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology

For many organisms, odor plays a major role in localizing resources such as potential mates, conspecifics, food, and oviposition sites. Nevertheless, odor-source localization is a difficult task for several reasons, not the least of which is the turbulent and unpredictable nature of the odor plume. In addition, what we perceive as a single odor is generally many different odorant molecules creating an odor blend. We propose that complex odors can be considered unique "objects" separate from their individual components in much the same way we consider objects as composite images in the visual sense. This session will consider how insects perceive olfactory "objects", and how odor features are parsed from sensory input to behavior. We will follow the processing of complex odor information as a stepwise process from sensory fingerprints at the antenna to spatiotemporal modulation in the antennal lobe. Students will test their knowledge by constructing a virtual antennal lobe network of inputs, outputs, and interneurons to accomplish specific blend detection tasks.

10:30 - 10:45 Coffee

10:45 - 12:15: Discriminating bioassays—the Key to identifying semiochemicals and defining their behavioral role and ecological function

Ring T. Cardé
Dept. of Entomology, University of California, Riverside

Ecologists are mostly interested in the endpoints of behavior—outcomes of relevance to ecological processes. Did the mosquito bite a host? Did a male moth find a female? How many insects are in an aggregation, where is it located, and how long will it persist? Applied entomologists also tend to focus on outcomes. Can we use attractive lures for surveillance and sampling? Can we use formulated pheromone to interfere with mating? Chemists ask is the compound of behavioral relevance? Chemists prefer simple bioassays that provide rapid, binary outcomes, and generally avoid time-consuming, complex bioassays that mimic the full behavioral repertoire. Students of orientation are more interested in the journey than the destination: what maneuvers and sensory inputs enable an insect to find an odor-linked resource? Neurophysiologists delve into mechanisms of odor perception, processing, and central control of behavioral output.

Most chemical ecologists place orientation behaviors into two bins: attraction and aggregation. These are of course outcomes (arriving or arriving and then staying) and using these terms uncritically masks rather different orientation processes. Many assume that attraction is purely odor-guided in the sense that the responder is detecting differences in odor distribution and orienting up a concentration gradient. This can occur in still air when the insect is very close to an odor source, but at distances of a meter and beyond the odor gradient is too shallow for chemotaxis. Instead odor induces upwind movement as long as the responder remains in contact with the odor plume. Detection of wind direction for flying insects entails using visual feedback to gauge the insect's movement relative to the ground below (optomotor anemotaxis). In contrast, aggregation describes a reduction or cessation in locomotion—but it may initially involve conventional attraction to the aggregative site.

Many bioassay methods have been devised. A common assay (such a T- or Y-tube) that is useful in support of the identification of semiochemicals may tell us little about natural orientation processes—insects usually are constrained to walk upwind in such devices. Semiochemicals that can be discriminated in such tests may be equally attractive in a single-choice test and these compounds may prove inactive in the field.

The four-armed olfactometer is widely used in assays—it can measure aggregation, but the accumulation of insects in one odor field can involve either increased or decreased locomotion, and either attraction or simply random entrance into the odor field. Accumulation of insects in one odor field has been described as attraction and as arrestment.

Some of the bioassay issues of relevance are: physiological state (influenced by age, daily rhythms, environmental conditions and strain differences); prior experience (including sensitization, habituation, imprinting and conditioning). The assay itself involves questions of its duration; using direct observation or monitoring of movement with IR counters or electric nets; need for positive controls in choice tests; number of subjects per replicate (facilitation); and the similarity of assay arena to natural conditions.

12:15 - 14:00 Lunch

14:00 - 15:30: Coding and processing of olfactory information visualized by functional imaging in the insect antennal lobe

Silke Sachse
Olfactory Coding Group, Max Planck Institute for Chemical Ecology

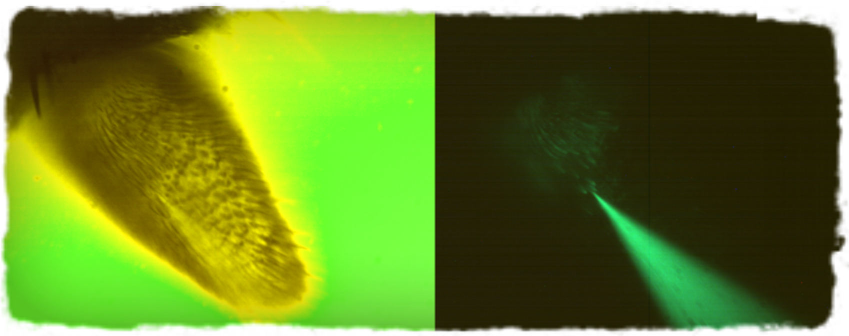
Optical recording techniques in insects allow visualization of how odors are represented in antennal lobe, the analogue to the vertebrate's olfactory bulb, leading to a specific odor perception. Several studies using intrinsic signals, voltage-sensitive dyes or calcium imaging have shown that odors are encoded as specific spatio-temporal "across-glomeruli" patterns. Each odor evokes activity in several glomeruli, whereas each glomerulus participates in the patterns of several odors in a species-specific manner. The olfactory system has therefore developed a strategy to encode the huge number of odors with a limited number of coding units. Although the different studies agree on how odors are encoded, different publications report contradicting results regarding the transformation of odor representations on the different processing levels within the antennal lobe. The lecture will summarize recent insights into olfactory coding strategies yielded by morphological and functional analysis of the different neuronal populations present in the insect antennal lobe and aim to find the link to odor-guided behavior. Moreover, optogenetic tools that are available in Drosophila will be introduced to selectively manipulate specific olfactory circuits.

15:30 - 17:00: Insect-robot hybrid system for understanding the neural basis of odor-source localization

Ryohei Kanzaki
Research Center for Advanced Science and Technology, The University of Tokyo

Insects display a surprising diversity of sophisticated behaviors adapted to the environments they populate, generate by information processing in relatively simple nervous systems. Once released from their source, odor molecules float through the air in complex spatial and temporal patterns. Even under such adverse conditions, insects can trace and orient toward the pheromone of a mating partner. In order to understand the neural bases of adaptive behavior, we employ a strategy that tackles the question a multiple levels, from genes, single cells of the neural system to the actual behavior. To examine the neural basis of the behavior, we implemented a model of the neural circuit, and integrated it with a mobile robot. Moreover, in order to understand the dynamics of the neural circuitry, we have developed an "insect-robot hybrid system" in which the insect or an isolated insect brain controls a robot. By comparison between the hybrid system and the model of the neural circuit of the insect, we can continuously improve the insect-brain model until we obtain a full emulation and complete understanding of the mechanisms of adaptability in the insect brain.

17:00 - 19:00: Stammtisch



Single sensillum recording in an Or22a-Gal4; UAS-CD8-GFP *D. melanogaster* transgenic line under bright-field (A) and fluorescent (B) light. By S. Olsson.

Friday December 7, 2012

9:00 - 10:30: Manipulating gene expression to study plant-mediated ecological interactions: lessons from *Nicotiana attenuata*

Ian Baldwin
Dept. of Molecular Ecology, Max Planck Institute for Chemical Ecology

Next generation plant ecology is characterized by the efficient use of next generation sequencing methods. This includes the identification of genes and markers of potential ecological importance. However, when it comes to testing hypotheses about the function of particular genetic loci for ecologically relevant traits, the manipulation of expression of these loci remains the most efficient means of falsifying hypotheses about ecological mechanisms and gene function.

This lecture will provide an over view of the development of efficient stable and transient silencing systems and a micrografting procedure for a native tobacco plant, *Nicotiana attenuata*, and how they have been, or are being modified to allow for fieldwork. The advantages and disadvantages of both transformation systems will be described and discussed. Both procedures are highly constrained by legal and ethical considerations and their material requirements differ substantially as well. The lecture will also describe how both procedures have been extended to include not only the manipulation of plant genes but also the genes of hetertropic ogranisms that consume the plant tissues, in particular, the Lepidoptera, in which standard procedures of RNAi are challenging.

References:

Galis, I., Schuman, M., Gase, K., Hettenhausen, C., Hartl, M., Dinh, T. S., Wu, J., Bonaventure, G., Baldwin, I. T. (in press). The use of VIGS technology to study plant-herbivore interactions. In A. Becker (Ed.), Virus-induced gene silencing: Methods and protocols.

Gase, K., Weinhold, A., Bozorov, T. A., Schuck, S., Baldwin, I. T. (2011). Efficient screening of transgenic plant lines for ecological research. Molecular Ecology Resources, 11(5), 890-902. doi:10.1111/j.1755-0998.2011.03017.x.

Kumar, P., Pandit, S. S., Baldwin, I. T. (2012). Tobacco Rattle Virus vector: A rapid and transient means of silencing *Manduca sexta* genes by plant mediated RNA interference. PLoS One, 7(2): e31347. doi:10.1371/journal.pone.0031347

Bubner, B., Gase, K., Berger, B., Link, D., Baldwin, I. T. (2006). Occurrence of tetraploidy in *Nicotiana attenuata* plants after Agrobacterium-mediated transformation is genotype specific but independent of polysomaty of explant tissue. Plant Cell Reports, 25(7), 668-675. doi:10.1007/s00299-005-0111-4. [ITB165]

Saedler, R., Baldwin, I. T. (2004). Virus-induced gene silencing of jasmonate-induced direct defences, nicotine and trypsin proteinase-inhibitors in *Nicotiana attenuata*. Journal of Experimental Botany, 55(395), 151-157.

Krügel, T., Lim, M., Gase, K., Halitschke, R., Baldwin, I. T. (2002). Agrobacterium-mediated transformation of *Nicotiana attenuat*a, a model ecological expression system. Chemoecology, 12(4), 177-183.

Fragoso, V., Goddard, H., Baldwin, I. T., Kim, S.-G. (2011). A simple and efficient micrografting method for stably transformed *Nicotiana attenuata* plants to examine shoot-root signaling. Plant Methods, 7: 34. doi: 10.1186/1746-4811-7-34

10:30 - 10:45 Coffee

10:45 - 12:15: Evaluation and Farewell

Drosophila melanogaster.
Copyright: Bill S. Hansson, MPI chem. Ökol.

Poster Abstracts

Poster Abstracts

#1: Electroantennogram response of two major noctuid pests to “topping-mediated” VOCs emitted by cotton plants

1 Mélanie Marchand, 2 Laurent Dormont, 2 Bertrand Schatz, 3 Idrissa Téreta, 1 Alain Renou & 1 Thierry Brévault

1 CIRAD, Montpellier, France
2 CEFE, Montpellier, France
3 IER, CRRA, Sikasso, Mali

Field experiments conducted in Mali showed a reduction of bollworm infestation on cotton plants topped ten days after the onset of flowering, as well as on non-topped neighboring plants. The purpose of this study was to test the hypothesis that manual topping could affect the profile of plant volatile compounds (VOCs), which may in return affect moth behavior. We compared the emission of VOCs from topped and non-topped plants by SPME and GC-MS techniques. We identified five “topping-mediated” VOCs, emitted in larger amounts by topped plants 4-16 days after topping for α-pinene, β-pinene, myrcene and *p*-cymene, and 8-32 days after topping for trans-caryophyllene. The concurrent increase of these VOCs from non topped neighboring plants also suggests partial induction. Electroantennography on gravid *Helicoverpa armigera* and *Spodoptera littoralis* females (Lepidoptera: Noctuidae) showed that moths can detect topping-mediated VOCs. Behavioral tests should be conducted to evaluate possible deterrence for egg-laying females.

#2: Transcriptional regulation of plant defense responses by the MYC2 and MYC2-like transcription factors in *Nicotiana attenuata*

1 Melkamu G. Woldemariam, 1 Ivan Galis & 1 Ian T. Baldwin

1 Max Plank Institute for Chemical Ecology, Department of Molecular Ecology, Jena, Germany

In their natural environment, plants are exposed to various abiotic and biotic stresses that influence their survival or fitness. Attack from herbivores of wide feeding habits and preferences exemplify biotic pressure that plants deal with. In response, plants developed direct and/or indirect defense responses; the production of toxic or anti-nutritive compounds (E.g. nicotine, trypsin protease inhibitors, diterpene glycosides, phenolamides) that negatively affect the growth and reproduction of attacking herbivores or attracting the predators or parasitoids of the attacking herbivores using volatile organic compounds. Production of these defense compounds is costly; hence their production needs strict regulation. One way in which defense responses are regulated is through attenuation of the defense responses by removing the active defense signaling molecules. Another way in which plants regulate defense responses is regulation of transcriptional responses by a wide array of transcription factors. MYC2 is a transcription factor that belongs to the bHLH family of transcription factors that regulate various physiological activities in plants. The MYC2, specifically, is reported to play a critical role in regulating genes involved in plant defense responses either directly or indirectly through controlling the transcription factors that control them. As a transcription factor controlling defense related genes, MYC2 has a central position in the JA signaling. In *N. attenuata*, we have found two MYC2 genes (and putatively named them as NaMYC2 and NaMYC2-Like). Using transcriptomic (microarray and qRT-PCR) and unbiased and targeted metabolomic approaches, we determined the regulatory roles of these transcription factors in *N. attenuata*.

#3: The effect of drought on peppermint stored and emitted terpenoids

1 Lenka Forkelová, 2 Sybille Unsicker, 1 Jan Muhr, 1 Susan Trumbore

1 Max Planck Institute for Biogeochemistry, Jena, Germany
2 Max Planck Institute for Chemical Ecology, Jena, Germany

Peppermint is an essential oil rich plant having its usage in food, cosmetic and pharmaceutical industry. Because of limited carbon assimilation during drought, plants change their resource allocation into secondary metabolites. Drought can thus influence plant resistance to biotic and abiotic stresses. By the means of GC-FID and GC-MS we studied the effect of drought on volatile and stored terpenoids in peppermint (*Mentha piperita* L.). Mild and severely drought stressed plants received for 30 days 50% and 20% of irrigation used for control plants. Decreased amount of irrigation caused 20% and 38% reduction of aboveground biomass of mild and severe stressed plants, respectively. The ^{13}C isotope signature of leaves was treatment specific and corresponded with individual drought levels. The C:N ration was significantly low in mild as well as in severe drought treatment. Drought caused significant decrease of total stored monoterpenes in severely drought stressed plants. Mild drought had no significant effect. No qualitative change in stored compounds in both drought treatments was detected. Emissions of monoterpenes were compound specific detecting decrease as well as increase in

emissions. Emissions of monoterpenes of control plants make up significant amount of stored compounds. Peppermint is a typical species which stores terpenoids in external structures (glandular trichomes) and makes the study of drought effect on terpenoids emissions challenging.

#4: An anatomical atlas of the tyraminergic/octopaminergic neuronal innervation patterns of the flight muscles of Drosophila.

1 Konstantin Lehmann & 1 Hans-Joachim Pflüger

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Most if not all larval and adult muscles in *Drosophila* are supplied by tyraminergic/octopaminergic neurons and these persist during metamorphosis. But it is unclear how the neurons change during the developmental stages. To investigate this a framework is required which ensures that the processes of plasticity which occur during the development are not biased by the inter subject variance. For this reason we created as a first step an atlas of the adult flight muscles with their associated neurons. It is not so complicated to create an atlas (or standard) of a rigid and relatively compact body like a brain/ganglion. It is, however, more delicate to create an atlas of multiple bodies which are flexibly connected to each other like multiple muscles with their associated neurons. This situation occurs in the case of *Drosophila* flight muscles with their respective neurons. Thus, we created a process chain which minimizes the variance related to the dissection and the further immunohistochemistry steps. After scanning with a confocal microscope we applied image processing techniques like stitching and different filters to improve the quality of the image stack. For the alignment which is the first step for creating an atlas/standard we used the 'principal skeleton' technique. The result was passed on to a grey value registration which computes the standard. The next step will be to create such standards for the various pupal development stages.

#5: Olfactory adaptations: insights into the *Drosophila erecta-Pandanus* association

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Many insects primarily rely on the sense of smell to orient within their environment. The olfactory systems of insect species should, therefore, reflect adaptations to specific hosts. The vinegar fly *Drosophila erecta* is remarkable for its specialization for feeding and breeding on ripe fruits of the screw pine tree *Pandanus* spp. in gallery forests of west central Africa. *D. erecta* belongs to the *melanogaster* species subgroup, which originated in tropical Africa. Besides a close relationship, the subgroup shows a considerable interspecific variation - ranging from extreme specialists to generalists and from endemic to cosmopolitan species.

My research addresses central questions about the adaptation of insect olfactory systems in response to host characteristics - specifically, whether the specialization towards its host fruit has caused changes within the olfactory system of *D. erecta*. To study effects of host specialization, we here compare several co-occurring *melanogaster*-subgroup sibling species with different host specificity and ecology. By combining methods of different fields, e.g. ecology, electrophysiology, immunohistochemistry and behavioural biology, we were able to explore the olfactory system of *D. erecta* and suggest that the *D. erecta-Pandanus* association is a result of an insect-host adaptation. This project was supported by the Max Planck Society.

#6: Attraction of codling moth to yeast: facts and hypotheses

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Codling moth is a key pest of apple causing severe loses. So far insecticides have a key role for its management, however due to their adverse effects, it is essential to develop alternative control strategies. In recent years great progress has been made in the field of insect olfaction. Identification of chemical volatiles that guide insects to food sources and oviposition sites is a current urgent research challenge. In this prospect a lot of effort has been done concerning host plant interactions in codling moth in order to identify the chemical cues that mediate codling moth attraction to apple, by studying apple volatiles only. Insect-yeast interactions have been known for decades, however, only recently has been shown that codling moth is closely associated with yeasts of the genus *Metschnikowia*. More specifically, it has been shown that codling moth is associated with specific yeasts that constitute an essential part of the larval diet since they strongly synergize to larval development and survival on galleries of apples. Furthermore, it has been demonstrated that egg-laying females of codling moth sense and respond to yeast volatiles. However, not all yeast species are attractant to codling moth since they emit different volatile compounds. Moreover, mating condition may also alter attraction of adults of codling moth as well as a tripartite relationship between moths, plants, and yeast. Yeast is expected to influence more life-history traits of codling moth and to play a crucial role in host finding.

#7: Interactions between the specialist herbivore *Bruchus pisorum* & its host *Pisum sativum* L: Sources of resistance in *P. sativum* accessions to *B. pisorum*.

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The pea weevil, *Bruchus pisorum* (L.) (Coleoptera:Bruchidae) is a major insect pest of field pea, *Pisum sativum* L. in the world. With the objective of identification of sources of resistance, a total of 600 *P. sativum* accessions were screened for the resistance to *B. pisorum* in three field sites. The result showed that there was a variation in numbers of eggs laid on the pods of field pea accessions tested. Mean number of eggs per pod ranged from 0 to 21.8. Among the accessions, 41 at (Ebinat), 46 (Liben) and 160 (Sekota) had the least (0.1 to 2.0) number of eggs per pod when compared with the number of eggs laid on other accessions. This may indicate that these accessions were less preferred for oviposition. On the other hand, in all of the study sites, most of the accessions had 2.1 to 6.0 eggs per pod. The highest numbers of eggs were recorded on few accessions at Liben site. Accessions with no and lowest number of eggs per pod probably suggesting a non-preference to ovipostion /antixenosis component of resistance to pea weevil in field pea accessions. Furthermore, seed damage analysis is undertaking in order to classify the accessions based on the level of resistance, and to identify sources of resistance to *B. pisorum*. Preliminary results showed that, there is considerable variation in the level of seed damage among the accessions tested, which further depicts potential sources of resistance in *P. sativum* accessions to *B. pisorum*. Future research work will focus on identification of mechanisms of resistance, and studies on the role of semiochemicals in the interactions of *B. pisorum* and its host plant.

#8: Chemical communication in Ithomiine Butterflies

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Insects communicate and perceive using multiple modalities: chemical, visual, and tactile. Depending on the abiotic and biotic environment different modalities will be more or less informative. Ithomiines are a group of neotropical butterflies uniquely suitable for these experiments because they have extensive mimicry groups, groups of butterflies sharing an aposomatic pattern. Butterflies are known to rely on both visual and chemical cues in approximately equal measure. Mimetic butterflies that closely resemble other species are likely constrained in the reliability of the visual modality for conspecific identification and this may cause a shift in modality use in deciding to approach potential conspecifics. Ithomiines are unique among butterflies in forming multispecies aggregations that serve as a natural laboratory for interspecific communication; aggregation formation is likely mediated by interspecific chemical communication. I use behavioral experiments to elucidate the use of modalities within aggregations. To examine the communication space used within and between species of ithomiines I use chemical and neurobiology approaches. Ultimately, I am interested in the selection pressures on communication space within and between groups (species, genders, trophic levels) and the costs of communication.

#9: How to search in a turbulent environment? Suggestions from a hawkmoth flight

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Since the French Naturalist Fabre so beautifully described the flight of the male peacock moth towards its conspecific female, the odor-guided flight of male moth become one of the classical study systems in ethology and until now numerous models have been proposed describing the underling mechanisms of this searching behavior.
In spite of these efforts, the flight patterns of the male moth towards food sources, such as nectar providing flowers, have received relatively little attention. Utilizing a newly developed 3d tracking system we are now gaining more insights into the flower-odor guided flight of the hawkmoth *Manduca sext*a.
In our current project we are testing the flight behavior of *Manduca* towards the full natural flower odor of different *Nicotiana* species. Among the selected species some can be assumed to be highly rewarding, whereas others are of a much lower value to the moth. Using our 3D tracking system we thereby hope to further unravel the influence of the stimulus quality on the flight kinetics of *Manduca*.
Neurophysilogical work on odor detection in *Manduca* indicates that the detection of plant volatiles might be achieved by a system very different to the one detecting female pheromones. Coherent with this work, our preliminary results also indicate different flight patterns towards a pheromone than towards flower odors. In light of this, we are ultimately interested in testing whether current models for odor-guide behavior such as anemotaxics, infotaxis or fluxotaxis can also be applied to male moths following the odor plume of different flower species.

#10: An *in vivo*-Atlas of the *Drosophila* Antennal Lobe based on Receptor Neuron Targeting

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One of the most important requirements for the analysis of *in vivo*-imaging dataset is the availability of a 3D-atlas of the neuropil of interest, e.g. the antennal lobe (AL) the first olfactory neuropil. The most commonly used atlases for the AL in *Drosophila*, are the ones generated by LAISSUE et al. (1999) and COUTO et al. (2005). They are based on either plain morphology or immuno staining.
These *in vitro* generated atlases have one general flaw making it difficult to utilize them *in vivo*. As the brain is dissected out of the head capsule, the antennal nerve is cut. Without any attachment of the nerve, the AL is lacking the tension caused by it. This in turn results in a modified positioning of the subunits of the AL, the glomeruli, in relation to each other.
To solve this we generated a fly that expresses dsRed as a direct fusion with synaptobrevin and therefore enables staining of the neuropile in the living fly. Performing two photon microscopy we could reconstruct a 3D-model of the *in vivo* brain and subsequently identify glomeruli.
The identification was supported by additional labeling of specific receptor GAL4 lines, incorporating ORs as well as IRs, with GFP in the background of the synaptobrevin-dsRed. These functional identifications of glomeruli together with the morphological ones provide a functional atlas as the basis for an *in vivo* characterization of frequently used ubiquitous GAL4 lines. Utilizing the same background labeling we mapped the *in vivo* condition of the most commonly used lines in olfactory research not only regarding their plain glomerular repertoire but also their arrangement for simplified assignment of odor evoked imaging signals.
Further it is now possible performing ultrastructural analyses of the intraglomerular distributions of different neuronal populations *in vivo* leading to a more comprehensive morphological understanding of the combinatorial AL map.

#11: Follow the odor plume or the path-integration vector? Homing versus foraging in desert ants

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Desert ants are equipped with a remarkable navigational toolkit that enables them to find their way successfully through the desert when foraging for food or heading back home to the nest. Path integration is performed continuously, nevertheless, *Cataglyphis* ants are able to use a variety of other cues for orientation. When returning to the nest or to a previously visited feeding site they perform path integration (PI) and finally follow the odor plume. In homing ants path integration dominates plume following. Ants respond to nest odors only when they are close to home, and - thereby - avoid becoming killed in a foreign nest. This is important insofar as plume following is not nest specific but ants pass foreign nest plumes during homing. However, we could show that the weighting of path integration and plume following is context dependent. In foraging ants plume following dominates path integration. Although path integration guides the ants to a learned feeding site, the ants respond to food odors independently whether they are close to the learned feeder or not. This might be beneficial as new food sources never pose a threat but enable the ants to shorten the foraging distances.

#12: Host selection, oviposition behavior and leaf traits of *Salix* spp. in a specialist willow sawfly

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Plant genotype often influences plant-phytophagous interaction by affecting insect attraction, acceptance and development. Host plant selection by females can be crucial for offspring survival. We evaluated the oviposition behavior of the specialist willow sawfly *Nematus oligospilus* (Hymenoptera: Tenthredinidae) on different genotypes of *Salix* spp. (Salicaceae). How oviposition is affected by leaf micromorphology, nutrient levels and secondary metabolites of the host plant is discussed.

Through choice and no-choice bioassays we analyzed host selection, larval performance and adult fecundity according to willow genotype. Leaf surface (adaxial/abaxial) and micromorphology were described. Total nitrogen, protein content, total phenolics, phenolic glycosides and salicin were quantified.

Results revealed that *N. oligospilus* strongly prefers to oviposit on *S. nigra* regardless of leaf side. Even though *S. viminalis* was the least preferred genotype, it showed better larval performance and higher adult fecundity. Leaf micromorphology analysis showed a tight association between the egg and the leaf. Leaf toughness was similar in *S. viminalis* and *S. babylonica*, and significantly lower in *S. nigra*. Total nitrogen and protein content were higher on *S. viminalis* and *S. babylonica*, while total phenolics and phenolic glycosides were higher and more diverse on *S. nigra*. Since salicin levels correlate with oviposition preference, this suggests a role of salicylates as oviposition stimulants.

Altogether, results suggest that oviposition preference may be related to lower leaf toughness and ease of injection of female saw-like ovipositor, and motivated by the presence of phenolic glycosides. Nitrogen levels may explain better larval performance and adult fecundity in *S. viminalis*. Thus, a balance among the different leaf traits determines the outcomes observed here.

#13: Herbivore-induced changes in floral olfactory traits contrast with floral visual traits in their effect on pollinator behavior

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Herbivore-induced defense in vegetative plant parts has been studied intensively, but whether herbivory similarly affects flowers and thereby pollinator behavior has rarely been considered. Jasmonates (JA), systemically mediating herbivore-induced reactions within vegetative plant parts may also have an effect on floral traits. We tested whether herbivory by *Aphis fabae* and JA treatment affect the olfactory and visual floral traits of *Vicia faba* and the behavior of pollinating bumblebees (*Bombus terrestris*). Bioassays with unscented flower dummies showed that showed that naïve foragers preferred dummies with larger spots and were able to discriminate minimal differences in spot sizes in learning experiments. Flight cage experiments with scented and unscented *V. faba* flowers revealed that naïve bumblebees highly rely on scent to locate flowers and are nearly unable to find flowers that do not emit flower-specific volatiles. Floral headspace volatile composition of aphid-infested and control plants differed, which was reflected in Y-maze olfactometer choice tests where bumblebees preferred scents of control plants over scents of aphid-infested ones. In contrast, bumblebees showed no preference when both floral olfactory and visual traits were offered in flight cage experiments. Instead their choice was positively correlated with melanin spot size. Thus herbivore-induced changes in floral olfactory traits may contrast with floral visual traits in their effect on pollinator behavior. JA treatment however, had no effect at all neither on floral traits nor on bumblebee behavior. In future studies we will examine changes in floral visual and olfactory traits in detail and investigate the effects of another guild of herbivores.

#14: Inferring from variation and covariation of leaf, flower, and fruit traits to the evolution plant-arthropod interactions

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Over the last decade, the understanding of complex plant-animal interactions has qualitatively improved thanks to the use of network analysis. Studies on ecological networks are mostly restricted to interactions at individual plant organs, e.g. flower-visitor or leaf-herbivore interactions. However, those actions do not occur in isolation. Different parts of plants require contrasting services: vegetative plant-parts avoid interactions with herbivores, flowers and fruits advertise interactions with mutualists, while antagonists should be deterred from these valuable tissues. Therefore, different organs evolved adaptations to its specific (potential) interaction partners leading to phenotypes with highly complex patterns of variation. Traits can vary individually or in concert with other traits resulting in a pronounced covariation maintaining certain functions, *sensu* phenotypic integration.
Here we introduce a study linking intra-specific variation of morphological, visual, and olfactory leaf, flower, fruit and seed traits from three plant species with their multiple interactions with arthropods, adopting a network approach. This data set will be complemented with evaluations of reproductive success of individual plants. We are planning to test (a) whether and how the interactions are shaped by traits and (b) whether (co-)variation results from adaptations to mutualists and antagonists or by developmental constraints. These results will further our understanding of the evolution of plant traits and their link to plant fitness via biotic interactions.

#15: Bees choose benzenoids, flies prefer terpenoids: floral scent bouquets determine flower visitor composition

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Flowers use visual and olfactory cues to communicate with their visitors. The existence of a diversity of insects interested in the consumption of nectar and pollen forces the plant to discriminate between mutualists and antagonists. One way of selecting the most beneficial visitors is the emission of floral scent bouquets that are able to function as both attractants and repellents, i.e. as floral filters. We selected two plant species *Achillea millefolium* and *Cirsium arvense*, the scent bouquet of the former is dominated by terpenoids, the latter by benzenoids. By applying scent extracts of one species onto flowers of the other species, we tested the effect of floral scents from different biochemical pathways on the visitor spectrum. Terpenoids applied onto flowers of *C. arvense* added a repellent function preventing honeybees and bumblebees from visiting the flowers. In contrast, benzenoids added to *A. millefolium* flowers occasionally attracted bees that usually do not visit *A. millefolium*. These results were confirmed in olfactometer trials testing the behavioural responses of naïve and experienced flower visitors. Effect of extracts was temporally limited, which was observed in the field as natural visitor spectra were re-establishing after 15 min and confirmed by GC/MS analysis. Our data clearly demonstrate that flower scents with their attractive and repellent properties have a pronounced impact on flower partitioning among visitors.

#16: Spatial representation of the olfactory output in *Drosophila*

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Finding food sources, good mating partners as well as oviposition sites and avoiding danger is essential for the fruit fly *Drosophila melanogaster*. This is relying on the sense of smell. Olfactory sensory neurons (OSNs), housed in sensilla on the antennae, express one type of odorant receptors each. The binding of an odor molecule to a specific OR evokes neuronal activity, which is transferred via the OSNs to distinct brain structures, so-called olfactory glomeruli, of the antennal lobe. Within the glomeruli synaptic connections to second order neurons (projection neurons, PNs) as well as interconnections between local interneurons take place. The latter is assumed to modulate the input signal via excitation and/or inhibition on particular synaptic terminals from OSNs to PNs. The modulated input signal is transferred via PNs to higher brain centers, like the mushroom body calyx and/or the lateral horn, leading to odor-guided behavior.

In the antennal lobe each OSN type innervates one corresponding glomerulus - creating a topographic map of odor reception. For further anatomical characterization of the innervation pattern in higher brain centers of inhibitory and excitatory PNs we used photoactivatable GFP. This method enables labeling of single neurons by irradiation of single somata or labeling of all PNs innervating a specific glomerulus. Of special interest are PNs innervating glomeruli that are predominantly activated by odors that elicit either aversive or attractive behavior. Photoactivation is used to generate a topographic map of these PNs innervating distinct or overlapping regions in higher brain centers.

This study is supported by the Max Planck Society, the BMBF and the IMPRS.

#17: Development and Evaluation of Push & Pull Strategies in Mosquito Control

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Push and pull strategies take advantage of the fact that insect pests use a variety of semiochemicals to locate mating partners, oviposition sites and hosts for bloodmeals. Through the use of both deterring and attracting stimuli, the abundance of insect pests can be changed by interfering with the ability of the target pest to find their resource ("push") and luring them to an alternative source where they are trapped ("pull").

A successful push & pull strategy in mosquito control could be based on volatile repellents in combination with alternative host cues, e.g. mosquito trapping systems. My approach involves the BG-Sentinel™ trap (BGS) as a pull component for *Aedes aegypti* in combination with spatial repellents, e.g. substances which work as *kairomone attraction inhibitors*.

Y-tube olfactometers were used to measure behavioural responses of host seeking *Ae. aegypti* towards a finger in the presence and absence of different test compounds. The most promising compounds reduced test mosquitoes´ attraction to the finger by more than 90%. However, results from olfactometer tests may not correlate well with field results, due to the confined volume and short distance from the point of odour release. A room test was designed to evaluate the most promising compounds under more realistic conditions. The set-up involved a simple push and pull situation, consisting of a repellent dispensing system and BGS trap, to protect a volunteer sitting inside a tent. Compared to olfactometer tests, the attraction reduction in room tests was lower but human landing rates could be reduced by up to 44%.

#18: Horizontal inter-colony transmission of the parasitic mite *Varroa destructor*

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During last three decades the honey bee parasitic mite *Varroa destructor* became present world-wide, heavily affecting honey bee populations, honey production and beekeeping. Horizontal inter-colony transmission of *V. destructor* is mainly by drifting of foragers bees. Furthermore, robbing is another way of varroa mites transmission that usually follows a lack of foodstuff availability in the field, being the invasion of weak colonies a likely alternative. During robbing a stress situation in the beehive due to fights between the individuals from different colonies take place, and the mites seem to abandon they original host to subsequently infest robbing bees which will later transport them to their colonies. The host-changing during robbing is a key point for the mite dispersion and may be mediated by chemicals compounds. In our study the question we seek to address is: What triggers *V. destructor* to switch host in order to leave the robbed colony? How does the transfer actually take place? Whit respect to this, we hypothesize that during robbing varroa mites perceive certain chemical compounds that triggers their host change. We aim to investigate volatiles compounds and epicuticular hydrocarbons in honey bees under stress conditions. Successively, we would be interested in testing the behavioral effect of those compounds in varroa mites.

#19: Herbivore-induced leaf-defenses deplete root carbon reserves and constrain - *Nicotiana attenuata* regrowth in a jasmonate-dependent and independent manner

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Leaf-herbivore attack induces systemic changes in plant roots. Until today, it remains poorly understood how these changes affect the plant's capacity for regrowth following herbivore defoliation. To study the impact of leaf-herbivory on root-mediated regrowth, we induced *N. attenuata* plants by applying *Manduca sexta* oral secretions to wounded leaves, then removed the whole shoots and measured growth, fitness and defense of the new shoots. Both in the laboratory and the field, we observed that wild type plants regrew less, had less flowers and were more resistant to *M. sexta* feeding after simulated leaf herbivory. We also observed a reduction in soluble sugars and starch content in the roots of induced plants. We therefore hypothesized that the herbivore-induced reduction in regrowth may be due to a depletion of carbon reserves in the roots following the increased investment into leaf defenses. To test this hypothesis, we used irAOC plants which are defective in jasmonate signaling and the induction of defensive secondary metabolites (1). We observed that induced irAOC plants did not suffer from a reduction of photoassimilates in the roots and regrew similarly than uninduced plants. Equally, we did not observe a reduction in regrowth in the irGAL83 line which constitutively allocates more photoassimilates to the roots (2). We therefore conclude that the JA-dependent depletion of root carbon reserves following leaf-induction has a negative effect on regrowth capacity and fitness of attacked plants. Our experiments suggest that belowground plant organs may be important mediators of growth-defense trade-offs in herbivore-attacked plants.

1. Kallenbach et al. 2012.PNAS 109 (24):E1548-E1557

2. Schwachtje et al. 2006. PNAS 103(34):12935-12940

#20: Evolution of CHCs profiles in cuckoo wasps and their hosts: is there evidence for chemical mimicry?

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The external waxy layer of the insects cuticle is mainly composed of non-polar hydrocarbons (CHCs). These chemically simple compounds serve several important functions: they do not only act as barriers against dessication and bacterial and fungal infections, but they also enable chemical communication both within and between species. Parasitic species may exploit these hydrocarbons to deceit their hosts using chemical mimicry: by mimicking the CHC profile of their hosts, parasites can avoid being recognized by them.

We aim to understand whether and how chemical mimicry has been a driving force in the evolution of CHCs profiles in a large taxon of brood parasites. Past research on chemical mimicry in parasitic species has mainly focused on closely-related host-parasite species pairs, thus being difficult to conclude whether the reason for CHCs similarities was due to phylogenetic relationships or selected by chemical mimicry. To better understand the influence of chemical mimicry, we propose studying and comparing the CHCs profiles of a species-rich group of cleptoparasites and parasitoids with their distantly related hosts. The diverse family Chrysididae (cuckoo wasps) appears to be an excellent choice. Previous studies have already shown that chemical mimicry is occurring in the cuckoo wasp *Hedychrum rutilans* and its obligatory host, *Philanthus triangulum* (Crabronidae) (Strohm et al., 2008). Although chemical mimicry seems to play a role in this species pair, whether this is a general strategy in the family remains to be elucidated.

Strohm, E., Kroiss, J., Herzner, G., Laurien-Kennen, C. et al. (2008). A cuckoo in wolves' clothing? Chemical mimicry in a specialized cuckoo wasp of the European beewolf (Hymenoptera, Chrysididae and Crabronidae). Front. Zool. 5: 2.

#21: Neurothology of oriental fruit moth olfaction, *Grapholita molesta* (Busck)

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We are working on the neuroethology of most destructive insect pest of peach, *G. molesta*. Olfaction is a very important for locating conspecifics and host plants. I aim to understand the mechanisms of pheromone-plant synergism by means of behaviour and electrophysiological studies to know if interaction occurs at ph-ORN or in PN's in the brain. The end goal is to develop environmentally-safe control methods. Antennal sensilla are being characterized by morphology (SEM) and the response of the ORNs located in them is tested with electrophysiology (SSR). SSR of the ORNs that respond to pheromone show that these ORN's are very specific and sensitive to pheromone compounds. Plant-volatile responding ORNs show varying degrees of specificity and sensitivity to plant volatiles. I will characterize the role of taste sensilla on the head appendages.

#22: The repellent properties: from the definition to the applications

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Laboratory and field studies have proven the effectiveness of using long lasting treated nets, such as bednet or agronet, to reduce the contact of vectors and prevent the risk of the transmission of pathogens. These nets are typically treated with a pyrethroid that has repelling, irritation and toxic effects to all insects. However, resistance of insect populations to these pyrethroids highlights the need for an alternative. The objective of my PhD project is to find repellent compounds for two study insects: *Bemisia tabaci*; and *Anopheles gambiae*, each a vector of the begomo vector of malaria viruses, respectively. The first step was to evaluate the repellent, irritant and toxic effects of 20 essential oils on *B. tabaci* adults and *An. gambiae* female adults in the laboratory. As essential oils are volatile aromatic compounds, their intended roles are to both attract pollinators or parasitoids and repel pests by the odor or anti-feeding effect. The most promising essential oils on both insects were found to be *Cymbopogon citratus*, *Cymbopogon winterianus*, *Cinnamomum zeylanicum*, *Cuminum cyminum*, and *Thymus vulgaris*. In a second experiment, the major compounds in the essesntial oils were identified by GC-MS. The effects of the identified compounds on both insect species were were further assessed in laboratory bioassays, using video tracking and EAG-GC. The first results on mosquitoes are given in the attached poster presented in E-sove 2012, 18^{th} conference of the European Society of the Vector Ecology, Montpellier, France, 8^{th}-11^{th} September.

#23: Chemical communication and neurophysiology of cooperation in social insects

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Social insects are key players in most terrestrial habitats. Their ecological success is based on an extraordinary cooperation in rearing offspring. The vast majority of colony members, the workers, refrain from reproduction in favour of the queen's progeny. However, they are able to lay unfertilised eggs that develop into males and they prefer to raise sons rather than brothers.

Bombus terrestris is a model organism to study this conflict over male parentage. In my PhD studies I employ chemoecological, endocrinological and neurophysiological methods to reveal mechanisms underlying the trade-off between social and selfish behaviour in bumblebee workers, addressing the following questions: How do workers determine the perfect time to lay eggs? Why do some workers reproduce whereas others remain sterile?

Studying the signalling function of nest wax, I found that wax lipids influence the reproductive decision of workers. In mirroring the social status of the colony, these substances trigger aggressive interactions and the development of worker ovaries. Further results indicate that physiological differences linked to task preferences prevent certain workers from becoming egg layers. In future investigations on the olfactory information processing of *B. terrestris* workers I hope to shed light on the role of olfaction in social insect cooperation.

#24: War in the cabbage patch; taking metabolomics into the field

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Plants have evolved survival mechanisms as dynamic as any behaviour observed in the animal kingdom. I am fascinated by the interaction between some of these mechanisms, such as plant secondary metabolites (PSMs) and insect herbivores. The wild cabbage, *Brassica oleracea*, provides an excellent study system for exploring ecological mechanisms maintaining the overwhelming diversity of PSMs. This relative of important crop species grows in fragmented populations in glorious locations throughout the UK and has well described genetics and biochemistry. Previous work indicates that insect herbivores exert differential selection pressures on the expression of compounds, such as glucosinolates, through species specific aversions and attractions. However much of this evidence comes from laboratory experiments or field studies which look at glucosinolates in isolation. To this end I am integrating approaches in functional biology and community ecology; techniques in metabolomics offer high-throughput and precise measurements of targeted compounds and the ability to investigate the whole plant metabolome. The application of these techniques to both wild and common garden populations has revealed more subtle variation in expression than previous results from allozyme and HPLC techniques has suggested. In further work I aim to explore the relationship between PSM variation, herbivore abundance and plant demography.

#25: Effects of cis-jasmone treatments on Insect Pests and Beneficial Insects Fauna of Wheat (*Triticumaestivum* L.)

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Wheat is one of the essential nutrients for humans. The most important factors that reduce the production of wheat are insect pests, diseases and weeds. Prevention of the losses caused by these factors is achieved by different control methods. Among the control methods chemical control so far has been the most widely used method. However, chemical control has many adverse effects on environment, human health, and destruction of beneficial fauna and degradation of the natural balance. Therefore, alternative methods to chemical control are needed. Utilization of inducing plants defence mechanisms and manipulation of natural enemies are new approach developed in recent years within this context. To this end, application of jasmonate derivatives e.g., cis-jasmone as a semio-chemical to reduce chemical control usage against insect pests seems to be a successful candidate of choice.

In present study, the differences of populations of some insect pests and beneficial insects between control plots and treated plots those are applied with three different doses of cis-jasmone (Dose1=25 g/ha; Dose2=50 g/ha; Dose3=100 g/ha) were studied by using three different sampling methods (Plant Sampling, yellow sticky trap, sweep-net sampling). There were significant differences in the numbers of European Wheat Stem Sawfly, *Cephus pygmaeus* L. (Hymenoptera: Cephidae), thrips (Thysanoptera: Phloeothripidae), lady beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and hoverflies (Diptera: Syrphidae) among cis-jasmone treated plots and control plots. Plants in control plots had higher numbers of thrips and *C. pygmaeus* than in cis-jasmone treated plots whereas higher numbers of *C. septempunctata* and hoverflies recorded in cis-jasmone treated plots.

#26: cis-Jasmone treatments affect sucking insect pests and their predators in cotton

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cis-jasmone is a plant volatile known to have roles as an insect semiochemical and in inducing plant defence. The resistance inducing effects and other effects (e.g., attractiveness of natural enemies) of cis-jasmone treatments in cotton were examined under field conditions. Effects of three doses of cis-jasmone (25g/ha, 50 g/ha and 100 g/ha) and control treatments (distilled water) on population fluctuations of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae); the cotton thrips, *Thrips tabaci* Lind. (Thysanoptera: Thripidae); the green leafhopper, *Empoasca decipiens* (Paoli) (Hemiptera: Cicadellidae) as pests and the green lacewing, *Chrysopa carnea* (Stephens) (Neuroptera: Chrysopidae) and the banded thrips, *Aeolothrips* sp. (Thysanoptera: Aeolothripidae) as predators were studied at three different cotton growth stages (6-8 true leaves (GS1), square bud (GS2) and blossom (GS2). Cis-jasmone effects varied among the cotton growth stages. There were less thrips and aphids at cis-jasmone treated plants in comparison to untreated control plants at GS1 and GS3 while had no such effect at GS2. The green leaf hopper densities were not affected by cis-jasmone treatment at all growth stages. The highest dose of cis-jasmone treatment (100 g/ha) attracted the higher number of green lacewing only at third growth stage treated (GS3). Both different cis-jasmone application doses and plant growth stages had no effect on the population of predatory thrips. The results were discussed in terms of cis-jasmone usage as a novel crop protection strategy. The preliminary results presented here, lead to carry out further comparative field studies involving other jasmonate derivates such as methyl jasmonate and jasmonic acid.

#27: Insect chemical ecology studies in Turkey

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Chemical ecology deals with the role of chemicals involved in the interaction of organisms with their environment, levels from molecular to ecosystems. In recent years there have been incredible developments and refinements in chemical analytical techniques for real-time analysis of volatile compounds and new ways of linking these to ecological bioassays.

While insects use a range of sensory cues, including vision and mechanosensory information, insect behavior and ecology is largely governed by chemical signals. Volatile organic compounds as well as contact chemicals have key roles in intraspecific interactions such as mating and resource competition, as well as interspecific interactions, such as host and food finding. Understanding the chemical interactions, and understanding of how insects perceive and process chemical information, requires sensitive analytical methods to quantify and identify relevant chemical compounds.

Currently, pheromone studies seem to be at the forefront among the other chemical ecology studies and pheromones are used commonly in insect pest management and registered particularly against lepidopteran pests in Turkey. The primary uses of insect pheromones are for monitoring, mass trapping, mating disruption, or lure-and-kill of insect pests populations.

Past Experience:

Using pheromones lures in pest management: Male of *Pseudaulacaspis pentagona* (Hemiptera: Diaspididae) monitored by pheromone traps to find number of generation per year and to determine proper period for augmentation and conservation of effective parasitoid, *Encarsia berlesei* (Hymenoptera: Aphelinidae) in part of PhD dissertation (in preparation).

Chemical Ecology of Parasitoids: Previously attended a summer course entitled "Chemical Approaches to Parasitoid Behavioural Ecology " Nottingham, United Kingdom, September 1-5, 2008 funded by ESF [Behavioural Ecology of Insect Parasitoids - from theoretical approaches to field applications (BEPAR)]: The Summer School addressed the problem by providing an overview of key tools used in chemical ecology of insects with examples of how they can be applied to behavioral and ecological studies. Typically, a seminar/lecture session was followed by a practical exercise and demonstration. Practical session comprised real-time analysis of volatiles of female-female contests for hosts in bethylid wasps (Hymenoptera: Bethylidae) by APCI-MS (Atmospheric pressure chemical ionisation–mass spectrometry).

Present Status:

I am now holding a permanent assistant professor position at Ordu University, Faculty of Agriculture, Department of Plant Protection, Entomology subdivision in Black Sea Region of Turkey. My current interests involved pests and natural enemies of kiwifruit, hazelnut and maize.

Future Prospects:

Study whether synthetic commercial pheromones of pest could work as a kairomonal stimulant and positively attract parasitoids or other non-target organisms by field studies

Learn and improve using chemical analytical techniques and designing experiments for parasitoid behavioral ecology studies via opportunities of postdoctoral research or as a visiting scientist.

#28: Population divergence through female choice in mason bees

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Vibrational and odor signals commonly play an important role in mating [1, 2]. These signals used during mating can play an important role in population divergence and ultimately speciation [3]. In a previous study we discovered that vibrations, odor and the relatedness with each other play a role in female choice in *Osmia rufa* [2].

To see if odor and vibrations also play a role in population and species divergence we investigated two subspecies of *O. rufa* using bees from England and Germany and from the supposed hybrid zone in Denmark [4]. We also used bees of the closely related sympatrically occurring sister species *O. cornuta*.

Our results clearly showed that *O.cornuta* differs significantly from *O. rufa* in both vibrations and odor. Furthermore we found significant differences in the frequency and modulation of vibrational signals as well as the odor between the bees from Germany and Denmark with the bees from England lying somewhere in between. This is surprising since we expected the two different subspecies of *O. rufa* to differ significantly, with Denmark lying in between. However we believe that this might be due to character displacement in Denmark, meaning that in sympatry, the bees have to differ more than in allopatry.

Based on these results we conducted bioassays to further investigate how vibrational signals are used by the bees. For these we used an interesting new technique involving magnets and an inductor to experimentally change the bee's vibrations.

1. Hill, P.S.M., Vibrational communication in animals. 1st ed2008, London, England: Harvard University Press. 261.
2. Conrad, T., et al., Female choice in the red mason bee, *Osmia rufa (L.)* (Megachilidae). Journal of Experimental Biology, 2010.
3. Coyne, J.A. and H.A. Orr, Speciation 2004, Sunderland, MA: Sinauer Associates.
4. Peters, D.S., Systematik und Zoogeographie der west-paläarktischen Arten von Osmia sstr, Monosmia und Orientosmia. Senckenbergiana Biologica, 1978. **58**: p. 287-346.

#29: Microbial symbionts and their functions in fungus culturing beetles

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Agriculture by insects evolved once in ants and termites and nine times in weevils, the ambrosia beetles. All of them farm specialized fungi for food, which is managed in cooperative societies, in which planting, protecting, cultivating and harvesting of the crops are shared by several individuals. Fungiculture apparently always evolved in combination with sociality of the host. The reason for this is unclear because we currently do not understand the mechanisms of social fungus-gardening behaviors. Most likely, they involve “microbial helpers” of the beetles.

In my project at the MPI-CE I will give first insights into the maintenance of fungus agriculture by ambrosia beetles and their associated microbiota. Together with a team of collaborators, I will start with providing the first complete overview of the community of microbes (i.e. bacteria, yeasts and filamentous fungi) associated with two ambrosia beetle species using culture-dependent and independent methods. Subsequently, dominant microbes will be further studied to reveal (i) whether and where they are carried within or on the body of the beetles, and (ii) how they interact with the ambrosia fungi and with each other. My overall aim is to unravel effects of particular microbes on the beetles' fungal crops by combining controlled bioassays with molecular and visualizing techniques as well as microbiological techniques to cultivate beetle-associated microbes. Provided appealing interactions, I further aim to isolate and chemically identify growth enhancing/antibiotic substances either from beetle extracts or particular symbionts. In summary, this will give first insights in the mechanisms of beetle fungiculture.

#30: Vision and Olfaction in *Sesamia nonagrioides*. How does it work in the Plant Colonization Process?

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The Mediterranean corn borer, *Sesamia nonagrioides* Lef (Lepidoptera: Noctuidae), is one of the most important borer pests of maize crops in northeastern Spain. In order to select an oviposition site the gravid females use chemical, visual, mechanical and taste cues, this stimulates receptors which generate sensory inputs and finally behavioral responses. This thesis aims to study the extent in which variables such as host location behavior, interspecific and intraspecific pheromone interactions, maize volatile compounds, abundance of olfactory sensilla and the role of vision and olfaction, determine the host location process in *S. nonagrioides* gravid females. In order to achieve this we will use different lab techniques such as electrophysiology, wind tunnel assays, olfactometer, SEM and GC-MS. We have identified the behavioral steps taken by *S. nonagrioides* females during host location and we will relate these steps with the antennal and ovipositor morphology. In this context we will examine the types, density and distribution of antennal and ovipositor sensilla. Moreover we have evaluated the electrophysiological and behavioural response of mated and unmated females and males of *S. nonagrioides* to their own pheromone and to the pheromone components of *Mythimna unipuncta* and *Ostrinia nubilalis*. All results obtained until now will be compared and related to those obtained in future experiments in order to better understand the plant colonization process of *S. nonagrioides* females.These results will be important not only in a biological or ecological context but also for the implementation of novel techniques of integrated crop protection based on the host location knowledge.

#31: Semiochemical parsimony in *Leptopilina heterotoma* (Hymenoptera: Figitidae), a parasitoid in *Drosophila*

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Wasps of the genus *Leptopilina* (Hymenoptera: Figitidae) are solitary larval parasitoids of *Drosophila* flies. In four combined studies we show a fourfold function of (-)- iridomyrmecin in the species *Leptopilina heterotoma*.

Female *L. heterotoma* produce (-)-iridomyrmecin in a cephalic gland and release it upon encounter with potential predators. Bioassays show that iridomyrmecin has a strong repellent effect on ants and that stereoisomers of iridomyrmecin differ in their repellent properties. Specifically, ( ) iridomyrmecin repelled the ants significantly longer from potential food items than (+) iridomyrmecin, (+) isoiridomyrmecin or ( ) isoiridomyrmecin. Males of *L. heterotoma* produce only the less effective (+) isoiridomyrmecin for defense. This suggests a second function of iridomyrmecin in the sexual communication of Leptopilina. Bioassays indeed show that ( ) iridomyrmecin is the major component of a iridoid mixture that attracts males, i.e. the female sex pheromone. Further investigation revealed that ( ) iridomyrmecin is also critical in eliciting male courtship behaviour. Lastly, we are able to show that ( ) iridomyrmecin is also used as a spacing pheromone by female wasps to detect and avoid host patches which are already exploited by conspecific or congeneric females. The fourfold function of ( ) iridomyrmecin in defense, sexual communication and host patch selection is an excellent example for the economic use of costly chemical messengers by insects, commonly referred to as “semiochemical parsimony”.

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#32: Comparative genomics of chemosensory repertoires in *Drosophila suzukii*

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Although the structure and the biology of chemosensory repertoires is well studied, little is known about their role in evolution and adaptation to new ecological niches. We address this problem by using as a model *Drosophila suzukii*, an emerging pest of grapevine that has the unique feature of laying eggs and feeding on fresh fruits, while most other Drosophila feed only on rotten fruits. The reason behind this innovative behaviour may lie in the repertoire of *D. suzukii* chemosensory proteins. It is therefore key to explore both gustative and odorant receptors and proteins in suzukii to understand the complex nature of how its antennae responds to fresh fruit odors. Comparative analysis of our recently sequenced *D. suzukii* trascriptome against that of other Drosophila shall reveal possible gene expansion and/or signature of molecular adaptation to odours emitted by ripening fruits. Phylogenomic approach will be employed to understand the evolution of the chemosensory repertoires.

#33: A not so pleasant bouquet: Leaf-cutting ants learn to reject *Vitis vinifera* ssp. *vinifera* plants with induced volatile plant defences

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Leaf-cutting ants (LCA) are dominant herbivores of the Neotropics as well as important pests, causing crop failures in many agricultural regions. Their foraging ecology and patterns/ mechanisms of food plant selection have received considerable research attention. Recent studies have documented LCAs to exhibit a delayed rejection of previously accepted food plants following treatment with a fungicide, making them unsuitable as substrate for their symbiotic fungus. We investigated whether the same rejection mechanism occurs when plants induced their chemical defense. We combined GC-MS analysis of volatile emissions with dual-choice bioassays with naïve and experienced LCA colonies (*Atta sexdens* L.). Ants were given the choice between untreated control plants and jasmonic acid induced test plants of *Vitis vinifera* ssp. *vinifera*. The results of the chemical analysis clearly indicated the emission of a characteristic set of herbivore-induced volatile organic compounds from JA-induced plants. The choice assays demonstrated that naive colonies showed indifferent foraging behaviour towards treatment and control plants, while experienced workers rejected the JA-induced plant. This indicates that *Atta sexdens* foragers learned to avoid VOC-emitting plants, which are harmful to their symbiotic fungus due to fungicidal components. Our findings provide first evidence that avoidance learning occurs in plants emitting defensive volatiles.

#34: Insights into the complex biology of an invasive Drosophila pest, *Drosophila suzukii*

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Drosophila suzukii, an invasive polyphagous pest on fruits native of East Asia has recently invaded western countries. Apart from the major damage caused by the larvae feeding on fruit flesh, the unique serrated ovipositor of the female encourages secondary microbial infection after piercing the skin of the fruit (Molina, 1974) and is considered a main threat to fruit production. *D. suzukii* presence in Europe is spreading rapidly and has now been reported in ten countries. Through morphological, behavioural, and electrophysiological studies, as well as genomic analysis we aim to identify shifts that accompanied niche differentiation in this species. Morphological studies include imaging different parts of male and female *D.suzukii* using Scanning Electron Microscopy (SEM) in comparison with *D.melanogaster*, the universally studied species. We tested the olfactory response of *D.suzukii* adults to its host plant volatiles using a Y-tube olfactometer and the results show strong attractiveness by both sexes. Electrophysiological experiments were performed using Gas Chromatography Electro Antennal Detector (GC-EAD) for fruit volatiles collected using Headspace collection method and the profile of volatiles eliciting electrophysiological response in the adults have been identified using GC-MS. These identified volatiles were again tested using synthetic compounds for the confirmation of their activity. The future assays will be performed with the identified compound/s in single or in blend of synthetics using Single Sensillum Recording (SSR) with an aim of developing a semio-chemical which has a potential for use in environmental-safe control of this pest.

#35: Electrophysiological and behavioural responses of Grapevine Moth *Lobesia botrana* to odours of the non-host plant *Perilla frutescens*

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Lobesia botrana is a major pest of grape worldwide. *L. botrana* kairomones have been extensively studied but their effectiveness for control purposes is negatively affected by the overlapping background odours in the vineyard. Thus, behaviourally active compounds from non-host plants may represent an interesting alternative for control.

Substances of food plants origins, i.e. from capsicum, garlic, pepper, mint, are known to activate specific receptors across species and phyla, giving the so called somatosensory sensation. These plants have also been used in agriculture for their known ability to interfere with insects and nematodes. Among those plants, *Perilla frutescens*, native of Asia, was shown to strongly activate human Transient Receptor Potential (TRP) channels, which are also expressed in insect antennae.

We therefore screened the biological activity of metabolites isolated from *P. frutescens* on the olfactory system of *L. botrana*.

Electrophysiologically active compounds released from 3 different *P. frutescens* chemotypes were identified by gas-chromatography coupled with electroantennography. In a dual choice oviposition test based exclusively on olfactory cues, females showed a preference for the odours released by a *Perilla* variety which profile is dominated by a C8 aldehyde, even in presence of the odour bouquet of grape bunches. Future molecular, physiological and behavioural studies will focus on the mechanisms of action of *Perilla* compounds on insect senses.

#36: Plasticity in olfactory-guided behavior of *Drosophila melanogaster*

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Fruit flies are, like most other insects, heavily dependent on their sense of smell when locating food resources, potential mates, or oviposition sites. Out of countless odors it is crucial for them to respond to only the small fraction relevant for survival and reproduction. However, the relevance of an odor is not only determined by the identity of the odor in question: its attractiveness can depend on environmental conditions such as light and temperature, or be affected by the internal state of the fly. Many factors can potentially induce physiological changes in an insect, and in order to minimize energy use and optimize survival it is important to modulate behavioral responses to odorants accordingly. The main focus of my research is to study how the internal state of a fly affects olfactory-guided behavior in *Drosophila melanogaster*, especially how fed and starved flies differ in their responses to odors. Working with this well-known model species offers a wide array of available methods to study olfactory-related plasticity, both for directly observing differences in behavior and for investigating how such differences are coupled with changes in the peripheral and/or central nervous system. An important tool for me will be to use optical imaging to elucidate if observed changes in behavior as a result of starvation are mirrored by changes in how odors are processed in the olfactory system of the fly.

#37: Plant volatiles might be involved in the niche partitioning between the Erythrina moths *Agathodes designalis* and *Terastia meticulosalis*

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Plants commonly use *volatile organic compounds* (VOCs) to communicate with other organisms such as herbivores, parasitoids and, even, themselves. Those emissions play an important role in the ecology of the plants and might be factors involved in the evolution of the herbivores. A recently described system involving *Erythrina herbacea* (Fabacea) and the "Erythrina moths" *Terastia meticulosalis* and *Agathodes designalis* (Lepidoptera: Crambidae) provocatively suggested that VOCs might be influencing the niche partitioning exhibited by these two species of moths. To test this idea further we used a chemical analysis to describe the differences in the blend of volatiles produced by the plants exposed to either of the two species of moths. VOCs were collected using glass chambers and Super Q adsorbent filters, followed by a subsequent analysis with gas chromatography. The results so far showed indeed that there are differences in the diversity of the compounds and their concentration. A data mining analysis with the Random Forest approach were used to classify volatile signatures and choose the most important compounds produced by the plants exposed to either of the two moths. To test the relevance of the observed differences in VOCs, we will continue a series of behavioral and ecological experiments that will give us a deeper understanding of the system and how VOCs might have had an important role in the niche partitioning of the Erythrina moths.

#38: Impact of leaf-miner insects on the primary metabolism of their host-plant: manipulating from the inside

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Endophytophagous insects, such as stem-boring, gall-forming and leaf-mining insects, live within plant tissues and feed internally. This life-style presumably provides adaptive advantages for the insect over other external-feeding modes by allowing access to most nutritional tissues while avoiding main plant defensive compounds. This selective feeding behavior can be reinforced by manipulating the plant physiology, as suggested by the autumnal formation of "green islands" around mining caterpillars in yellow leaves.

We study this so-called "nutrition hypothesis" on the biological system *Malus domestica* (Rosaceae) / *Phyllonorycter blancardella* (Lepidoptera: Gracillariidae). We characterized larval mouthparts by scanning electron microscopy and resulting leaf anatomy. Thanks to colorimetric assays, capillary electrophoresis and GC/MS, we quantified the alteration of proteins, starch, total soluble carbohydrates, main individual carbohydrates, protein-bound and free amino acids on green and yellow mined leaves. We also determined larval reserves (proteins, total lipids, total sugars, triglycerides, glycogene) by colorimetric assays on microplate to compare the intake target with the growth target of larvae.

We found that the leaf-miner larva has the ability to manipulate its host plant in order to generate a microenvironment with all the nutrient supply needed for its growth and its survival. Our results suggest that insects impact source-sink relationships through manipulation of Cytokinin levels leading to nutrient translocation or *de novo* synthesis. It also appears that endosymbiotic bacteria (*Wolbachia*) could be a nutritional-mutualist for the insect, in complement of their primary role played in the synthesis of Cytokinin phytohormones in mined tissues.

#39: Pheromone Mass Trapping Technology for management of Coconut black-headed caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) in Southern Karnataka, India

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Coconut black headed caterpillar (BHC), *Opisina arenosella* Walker is a defoliating pest of coconut in India, Sri Lanka, Burma, Bangladesh, Malaysia, Indonesia, Thailand and East Pakistan. In India, the BHC menace is being frequently noticed on large scale in south India causing considerable economic yield loss. The pest infestation is chiefly confined to the lower fronds, and the caterpillars feed by scrapping lower epidermis of leaflets. The infested fronds look like burnt up appearance and affected palms often take several years to completely recover. Present work was carried out on calling behaviour and male response towards sex pheromone and behavioural responses of male and female *O. arenosella* to female pheromone. Standardization of pheromone lure, dosage of the pheromone, trap type, trap installation height, trap color and trap density per hectare were also standardized. Studies on exploitation of pheromone traps as a surveillance and monitoring tool in IPM of *O. arenosella* were also conducted. Nearly 50 per cent of population reduction was found in mass trapping of male BHC moths.

#40: Studies on Chemical Ecology and Behavior of Coffee White Stem Borer, *Xylotrechus quadripes* Chevrolat (Coleoptera: Cerambycidae)

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Coffee is a native of Southwestern region of Ethiopia and a popular beverage consumed by 11% of the world population and ranks fourth after carbonated soft drinks (28%), milk (28%) and fruit juices (26%). It is popularly admired as "King of beverages". Damage caused by the coffee white stem borer (CWSB) is alarming, causing an economic yield loss upto 20%. It is known that CWSB preferentially attacks Arabica Coffee while Robusta variety is spared. Some preliminary studies have indicated that there may be some volatiles in the bark of Arabica that may be attractive to the females for egg laying. Hence, attempts are being made to identify the bark chemicals and isolate the active component, which may be useful in developing an oviposition attractant for either trapping females or for monitoring emergence.

During this study, we plan to work on host volatiles involved in host preference and on volatiles emitted by male & female CWSB. Volatiles from coffee plants of different varieties and from CWSB males & females are collected using volatile collection chambers. Attempts will be made to integrate these volatiles with the male pheromone which was identified earlier. Work is under progress and will be discussed during the presentation.

#41: Semio-chemical based intervention in *Eldana saccharina* (Lepidoptera: Pyralidae) infestations in sugarcane: Identification of active plant volatiles detected by the host and its parasitoids

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In its indigenous South African wetland sedge hosts (*Cyperus* spp.), the sugarcane borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae), is attacked by nine species of indigenous parasitoids and one indigenous pathogen with recorded parasitism of 45 %. Even though *E. saccharina* has been a pest in South African sugarcane since the 1940's, its parasitoids have not been found in sugarcane. In Kenya, it has been shown that maize plants attacked by the crambid stalk borer *Chilo partellus* emit volatiles that the braconid *Cotesia* spp. parasitoids respond to. We hypothesize that, whereas *Cyperus* spp. under herbivore attack release a volatile signal that attracts parasitoids, sugarcane, for unknown reasons, fails to produce this "SOS" signal. In this study we will determine which cues emitted from infested and uninfested host and non-host plants, as well as *E.saccharina* frass, used by selected parasitoids in host searching. Volatile emissions from the plants will be sampled using different dynamic headspace collection methods (thermodesorption, solvent extraction), and analysed via GC-MS coupled with electroantennogram detection. To determine the responses of *E. saccharina* and its parasitoids to the various compounds, active compounds will be subjected to olfactometer, cage and field bioassays.

#42: Induced Plant defense in *Cicer arietinum* L. in Response to a Chewing Insect *Helicoverpa armigera*

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Induced plant defense reinforces adaptive response during subsequent insect attack. To indicate the exhibition of induced defense in *Cicer arietinum* L. against a polyphagous insect *Helicoverpa armigera*, we monitored the effects of mild insect infestation on subsequent larval feeding behavior using focused bioassays comparing dispersal percentage and growth of larvae. Larvae released on elicited-plants had decreased larval performance and reduced preference, demonstrating the central role of induced plant defense against herbivory. Similarly, wounding and exogenous application of signaling molecules also affected larval growth and feeding behavior. To further understand the molecular basis of induced plant defense in chickpea, suppression subtractive hybridization (SSH) and macroarray approaches were combined to identify the potential important genes involved in the process. Of the 63 unigenes selected for further analysis, 29 genes expressed differentially when *Helicoverpa* feeding and wounding responses were compared. Profiling was also carried out with plants treated with methyl jasmonate (MeJA), salicylic acid (SA) and ethylene (ET) which revealed that most of the *Helicoverpa*-induced transcripts were MeJA and ET regulated. A set of transcripts has been identified, which have significantly increased expression and probably contributes to induced chickpea defense responses against *H. armigera*.

#43: Development of a high throughput cell-based assay for characterizing lepidopteran olfactory receptors

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The development of rapid and reliable assays to characterize insect odorant (ORs) and pheromone receptors (PRs) remains a challenge for the field. Previously insect ORs and PRs have been functionally characterized *in vitro* through expression in *Xenopus* oocytes, Sf9 cells and HEK293 cells. While these approaches have succeeded, these systems have inherent characteristics that prohibit them from being used in high throughput formats. We have developed an assay system whereby we can functionally characterize insect ORs and PRs in 96-well plates using a fluorescent spectrophotometer.

We began by making a T-REx HEK293 cell line capable of regulating the transcription of genes from plasmids containing the "tetracycline operator" promoter sequence. After confirmation of Tet-Repressor function, this cell line was stably transfected with the *Epiphyas postvittana* OR co-receptor (EposORCO). The cell line was then single-cell sorted and resulting clones were evaluated for inducible EposORCO expression by RT-PCR and western blot and localization by immunofluorescence. Receptor function was also confirmed by measuring calcium flux in response to the ORCO agonist, VUAA1. The isogenic cell line with the best inducible expression and function of EposORCO was chosen for subsequent expression and characterization of *E. postvittana* ORs and PRs.

To date we have identified 72 putative olfactory receptors from genome and transcriptome libraries of *E. postvittana*. Quantitative RT-PCR analyses have revealed a subset of these genes that display male-biased antennal expression, making them likely candidates for being *E. postvittana* PRs. Here we report the responsiveness of one of the male-biased *E. postvittana* receptors to the moth's sex pheromone components to demonstrate the utility of the system.

#44: Differentiation of the ant genus *Tapinoma* from the Mediterranean Basin by species-specific cuticular hydrocarbon profiles

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Correct species identification is a precondition for biological study, yet despite a long history of morphological investigations, the systematic position of many ant species remains unclear. Identifying cryptic or sibling species is essential because morphological similarity may mask great differences in behavior and ecology. Consequently, their reliable identification requires elaborate methods in multi-modal approaches such as high morphology analysis, DNA analysis or cuticular hydrocarbon (CHC) compositions. Like any phenotypic character, CHCs are reliable indicators of species identity. Here, we compared and identified cuticular hydrocarbon profiles of workers of several species of *Tapinoma* from Algeria, Morocco, Israel, France and Spain. We used the CHC profiles of workers to identify five *Tapinoma* species: *T. erraticum*, *T. israele*, *T. madeirense*, *T. nigerrimum* and *T. simrothi*. The species-specific hydrocarbon profiles were found to remain remarkably stable between Morocco and Northern France and between Israel and Algeria. They were not influenced by ecological factors such as vegetation type, soil and climate. In *Tapinoma* genus, cuticular hydrocarbon profiles were found to have a high diversity in CHC composition. These five identified *Tapinoma* species shared only three CHC. The findings from our work clearly show how using chemical facilitates the study of these species, by allowing precise differentiation and identification. The chemistry of the CHC bouquet is shown to be a good tool for taxonomists, being species-stable over thousands of kilometers.

#45: Mechanism of Olfactory Habituation in *Drosophila melanogaster*

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I have studied the neural and molecular mechanism of olfactory habituation in Drosophila. Such habituation could be relevant in wild, because by allowing flies to filter irrelevant and familiar odorants, habituation may allow animals to identify and navigate in response to more salient olfactory stimuli. Olfactory habituation a) occurs over short and long time scales in an odorant specific manner; b) occurs through a process of potentiation of inhibitory synapses of olfactory local interneurons forming onto olfactory projection neurons; c) involves multiple signaling pathways and complex transynaptic interactions. Using available genetic tools, olfactory behavior and functional imaging, we showed the requirement of different neurotransmitter in different set of neurons in olfactory circuitry in the process of habituation. Our study also addresses how the regulation of local translation by miRNA pathway regulates one type of habituation (LTH) but not the other (STH) indicating a potential distinction between two forms of learning[1,2].

This work led to my keen interest in inset olfactory behavior in the wild. I want to learn the methods and logic of chemical ecology: from isolation and characterization of chemical signals, to quantitative field biology. For example, plants respond to herbivore attack by releasing plant volatiles to attract insect predators of the herbivore, an indirect, but effective defense mechanism. Similarly plants attract appropriate pollinators and repel inappropriate ones. I am interested to study plant volatile induced alterations in insect behavior and in understanding how the insect "learns" in nature to generate and perform appropriate adaptive responses and actions.

1. Das, Sadandappa et al. Proc. Natl. Acad. Sci. U S A. 2011;108(36):E646-54
2. McCann, Holohan, Das et al. Proc. Natl. Acad. Sci. U S A. 2011;108(36):E655-62

#46: Plant-Plant Signaling in an Environmental Contex

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In the group of Chemical Ecology we are interested in plant-insect interactions and how volatile organic compounds (VOCs) emitted by plants behave in the atmosphere together with oxidative air pollutants. My research is focused on exploring questions related to plant-plant communication and the effect that environmental factors have on it. Questions such as what are the volatile signals that mediate this process? To what extent does tropospheric ozone disrupt this process? And, what is the ecological relevance of this communication?; are answered by conducting experiments under laboratory and field conditions. Recent experiments in the laboratory showed that Lima bean plants emit nitric oxide (NO) when damaged by spider-mites or mechanically. Hence, I am exploring a possible role for NO in plant-plant communication, either as a lone signaling molecule, or as part of a more complex signalling system. Field experiments are conducted in the Free Air Concentration Enrichment (FACE) facility that allows the localised enrichment of ozone concentrations relative to recorded ambient values. I use this facility to expose cabbage plants to moderately enhanced levels of ozone and to monitor the effects on plant-plant communication and the effects on VOC emissions by receiver plants in response to challenge by herbivores. I conduct egg-laying experiments using cabbage receiver plants from ozone-enriched and ambient air conditions, and adults of *Pieris brassicae* to evaluate how plants benefit from receiving volatile signals from their neighbours. The final goal of my research is to address mechanistic aspects of plant-plant communication and to understand how the ecosystem is responding and adapting to environmental change.

#47: Exploitation of early-herbivory-inducible innate defense traits in graminae in the management of stemborers through habitat diversification

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Lepidopteran stem borers are far the most damaging pests of cereal crops in sub-Saharan Africa causing crop loss of 20-80%. Efforts to control these pests using synthetic insecticides are constrained by development of resistance, limited efficacy, high costs to resource-poor smallholder farmers and elimination of its natural enemies, which has further worsened its pest status, hence the need to search for more viable alternative management strategies. Under natural conditions, plants have evolved direct and indirect defense strategies against attacking organisms. Directly, they produce toxins, digestion inhibitors and herbivore-induced plant volatiles (HIPVs) repellant to phytophagous insects. Indirectly, plants use HIPVs to attract natural enemies antagonistic to the herbivores. Previous studies suggest selective breeding of crops may have favored other traits like yield at the expense of innate defense traits. Our research interests are in the volatiles emitted by herbivore-infested plants and the tritrophic interactions with natural enemies of these herbivores in the earliest stage of attack, oviposition, in anticipation of larval hatching. The methodology will involve collection, GC-MS and electrophysiological analysis of HIPVs from oviposited maize seedlngs that are adapted to local agro-climatic conditions. Parasitoids' response to HIPVs will be tested using four-arm olfactometer bioassay. Oviposition preferences by moths will be carried out using oviposited and non-oviposited maize seedlings in oviposition cages. Plants possessing these traits will be selected for use in the environmentally friendly push-pull system for stemborer control. Additionally, local and systemic biosynthesis and production of plant secondary defense metabolites by plants upon oviposition by stemborers will be investigated.

#48: The role of allelopathy in *Heracleum mantegazzianum* invasion

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According to the novel weapons hypothesis allelopathy may facilitate invasions of exotic plants especially if the newcomer's biologically active compounds encounter nonadaptated native species (Callaway and Ridenour 2004).

Heracleum mantegazzianum (giant hogweed) is one out of 100 worst invasive species in Europe, drastically reducing the biodiversity and through its sap containing photodermatitic furocoumarins being dangerous even to people and animals (DAISIE 2008).

Although an increasing attention has been paid to allelopathy as an invasion mechanism during last decade, its role in *Heracleum mantegazzianum* invasion has been overlooked so far. It remains also unclear whether phytotoxicity in *Heracleum mantegazzianum*, if proved, is due to novel compounds, or rather due to compounds produced also by close native relative *Heracleum sphondylium*.

Here the root exudates collected of both *Heracleum* species, two other native plants and control were assayed for phytotoxic effects on germination and growth of native species common in the invaded biotops. Not only *in vitro* but also in soil. In addition to bioassays, attempts to indentify the active compound/s by means of chromatografic methods coupled with mass spectrometry are being developed.

The root exudates of *Heracleum mantegazzianum* proved to be phytotoxic, although inconsistent results were obtained. Moreover, they were not more phytotoxic than root exudates of *Heracleum sphondylium* and other native plants. In soils the effect of exudates was not phytotoxic or interacted with soil biota. To explain this variability we propose to assay root exudates from different *Heracleum mantegazzianum* populations separately and to accomplish the identification of active compounds.

1. Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2(8): 436–443.
2. DAISIE European Invasive Alien Species Gateway, 2008. *Heracleum mantegazzianum*. Available from: http://www.europe-aliens.org/speciesFactsheet.do?speciesId=21125 [Accessed 1st September 2012]

#49: Drought induced variations in the product of biogenic volatiles important for the "push-pull" strategy for the control of stemborers and their effects on the trophic interactions

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The push-pull strategy for the control of stemborers relies on the attractive and repulsive properties of the perimeter and inter-crop plants, respectively, to deceive gravid moths into laying eggs in perimeter plants as opposed to the cereal crop that will be protected. However, abiotic stressors such as drought are known to induce variations in the volatile emission profiles of plants. In particular, isoprenoid compounds produced in some plant taxa have been hypothesised to act as anti-oxidative agents, thus protecting plant tissue from oxidative damage as a result of drought stress. However, such changes in volatile emission may have consequent impacts on the herbivorous or parasitoid insects‘ oviposition decisions and host detection which may alter the trophic interactions important for the push-pull strategy. Using *C. partellus*, *B. fusca* and their parasitoids *C. sesamiae* and *C. flavipes* as model organisms, changes in their behaviour as a result of drought-induced alterations in volatile emission profiles of wild and cultivated grasses used in the push-pull will be investigated.

#50: Elucidation of semiochemicals from weaver ants and investigation of nutritional value of ant manure

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The objective of this PhD project is to elucidate the mechanisms behind ant-plant and ant-insect prey interaction in order to provide fundamental knowledge on the nutritive effect of weaver ant (*Oecophylla smaragdina* and *Oecophylla longinoda*) "manure" depositions on plant growth as well as fundamental knowledge on the chemical ant-ant and ant-prey signaling compounds (semiochemicals). The main hypotheses

are that the deposits of weaver ants contribute significantly with macro- and micronutrients to the growth of fruit trees, and that unknown signaling compounds of importance for ant-ant and ant-prey interactions are deposited together with the nutrients in the depositions. The project is carried out by use of advanced analytical chemical methods such as LC-MS (TOF, QTRAP, MSMS), GC-TOF-MS, ICP-MS, and LC-NMR.
The PhD project is part of the Ant-manure project - for more information see: www.ant-manure.dk.

#51: Interaction between plant growth promoting rhizobacteria and foliar feeding insects

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Plant Growth Promoting Rhizobacteria (PGPR) are a prime component of the soil microbial community and recent research shows that they can enhance the chemical defences of plants. Foliar secondary metabolites of plants have been widely studied for their role in defence functions against invading herbivores. However, the role of PGPR in determining the qualitative and quantitative aspects of secondary metabolites and thus the effects of bacteria-plant-herbivore tritrophic interactions remains sparsely explored. Therefore, it is critical to understand how and why PGPR affect foliar-feeding insects, in a microbial and chemical ecology framework. The aim of this study is to determine whether PGPR affect above ground herbivores, so that biotechnological approaches with field crop applications can be developed. Experiments have been undertaken in which the effects of different soil microbial enhancement methods are tested on several crops, the alterations in foliar leaf chemistry presented and consequences for above ground insects determined.

#52: One secretion to defeat them all? - The chemical defense of earwigs

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Earwigs, insects of the order Dermaptera, emit a malodorous secretion from paired abdominal glands when disturbed. Nevertheless, only two earwig species have been investigated regarding their chemical armature thus far. Therefore, we analyzed the defensive secretion of three dermapteran species using gas chromatography-mass spectrometry. In total, four alkylated 1,4-benzoquinones were identified; one thereof is hitherto known exclusively from opilionid exocrine secretions. Furthermore, species- and sex-specific differences in the chemical composition were observed. Previous studies suggest that the secretion is used primarily as repellent against various predators. As earwigs live in aggregations in narrow crevices and practice maternal care, e. g. by hibernating with their brood in nests belowground, their subsocial behavior and habitat preferences increase the need for enhanced defense mechanisms against co-occuring microorganisms and nematodes. To evaluate the antimicrobial activity of the different secretions, we conducted inhibition zone assays with gram-positive and -negative bacteria and two entomopathogenic fungi. Furthermore, the secretion was tested against the nematode *Caenorhabditis elegans*. Since the alkylated 1,4-benzoquinones were also detected in the headspace analyses of earwig aggregations, our results implicate that the defensive secretion is not only used to repel predators but also to disinfect the micro-habitat.

#53: Tracking Down the Semiochemicals in Vineyards – A New Mobile GC-MS-EAD to Investigate Mating Disruption Failures

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Mating disruption is widely utilised in ecological pest control. In viticulture it is used against *Lobesia botrana* and *Eupoecilia ambiguella*, which has led to a huge decline in the use of insecticides. As this method is increasingly applied in regions worldwide, wine growers and researchers also face problems in vineyards where the confusion via pheromone dispensers fails. In order to understand the circumstances that lead to these failures, analytical devices are needed that feature a high sensitivity for volatile organic compounds, short measurement time and portability. Therefore, portable electro-antennogram (EAG) devices have been invented, but they still produce non-comparable relative units and provide distorted results in the presence of non-target volatiles.

To avoid these disadvantages, we established an automated mobile device that comprises a needle-trap device (NTD), gas chromatograph (GC), mass spectrometer (MS), and electro-antennographic detection (EAD). Testing this setup with the *Lobesia botrana* main pheromone component 7,9-12Ac, we discovered a surprisingly low detection limit and a high dynamic range. Our first results indicate a new application strategy for the pheromone dispensers. A first series of measurements has been realized in vineyards and the data has been validated by cage-release and capture tests.

Different antenna holders allow the use in future projects addressing the perception of volatiles. Once adjusted to the morphological conditions of the insect antennae, the mobile NTD-GC-MS-EAD system can be used to monitor semiochemicals for other insect species in question.

Articles for Literature Review

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The evolutionary ecology of insect resistance to plant chemicals

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Understanding the diversity of insect responses to chemical pressures (e.g. plant allelochemicals and pesticides) in their local ecological context represents a key challenge in developing durable pest control strategies. To what extent do the resistance mechanisms evolved by insects to deal with the chemical defences of plants differ from those that have evolved to resist insecticides? Here, we review recent advances in our understanding of insect resistance to plant chemicals, with a special emphasis on their underlying molecular basis, evaluate costs associated with each resistance trait, and discuss the ecological and evolutionary significance of these findings.

Plant–insect interactions in a community context

Over recent years, considerable advances have been made in our understanding of the genetics of insect resistance to insecticides and genetically modified crops [1,2]. However, less is known about the genetic basis of insect resistance to plant chemicals that are present in their natural environment. The responses of insects to chemical insecticides have been informative models for studying molecular mechanisms of resistance. Do available data on the adaptation of phytophagous insects (see Glossary) to host plant defences compared with adaptation to insecticides suggest that similar or different mechanisms are at play? Although the chemical structure of some synthetic insecticides is comparable to that of some plant-produced compounds (e.g. pyrethroids and nicotinoids), the intensity of selection and the nature of insect resistance traits are likely to differ between these two types of selection pressure. Indeed, in their natural environment, insects face not only a range of plant defences that are heterogeneously distributed through time and space, but also selective pressures from predators, parasitoids and competitors. As a result, the degree and nature of their resistance to plant chemicals varies depending on the geographical distribution of plant phenotypes, the specificity of the plant–insect association and the local community composition.

Insect resistance to plant allelochemicals might interfere with their resistance to insecticides. For example, *Spodoptera frugiperda* caterpillars fed on particular plants become more tolerant to insecticides [3]; thus, understanding resistance mechanisms, as well as taking into account other ecological parameters, is important when predicting the spread of insecticide resistance in natural populations, and choosing the optimum strategy for managing pest populations.

From an evolutionary perspective, despite the supposed key role of the chemical 'arms race' in driving the coevolution of plants and insects, much research has focused so far on describing the diversity of plant chemicals and their effects on herbivores. Less is known about the multiple mechanisms evolved by insects to overcome these chemical defences (Table 1). These mechanisms include contact and ingestion avoidance, excretion, sequestration, degradation of the toxin and target-site mutation (Figure 1).

Here, we review recent advances in our understanding of insect resistance to plant chemicals and highlight two general trends: insects that are resistant to plant toxins usually combine several resistance traits (e.g. behavioural

Glossary

Allelochemical: chemical produced by an organism that is toxic to, or inhibits the growth of, other organisms; synonymous here with 'phytotoxin', 'plant chemical' and 'plant toxin'.
Aposematism: conspicuous colours, sounds, or other warning cues by which an organism openly signals itself as unpalatable to potential predators.
Autogenous defence: a chemical defence against predators that is synthesized by the insect metabolism, as opposed to the use of chemical compounds produced by the host plant (host-derived chemical defence).
Co-cladogenesis: the congruence of two phylogenies (e.g. plant and insect phylogenies) is an indication but not proof that the two studied lineages are under reciprocal selection (e.g. coevolution *sensu stricto*).
Constitutive resistance: resistance traits (e.g. the mutation of a particular enzyme or plant toxin receptor) constantly produced by an insect that reduce the negative effect of allelochemicals. Associated costs are fixed (independent of the probability of encountering a defended plant), resulting in decelerating fitness costs (relative to benefits).
Exuvia: the integument that is lost during the moult occurring between two growth stages in arthropods.
Induced resistance: traits that are produced or increased only after contact with plant allelochemicals. Associated costs increase with the probability of encountering a defended plant, resulting in accelerating fitness costs.
Metabolic resistance: a resistance mechanism involving detoxification enzymes that can catalyse the biotransformation of xenobiotics into metabolites that are less or non-toxic to the organism.
Phenolic compounds: plant secondary metabolites (including flavonoids, tannins, coumarins, phenolic acids, etc.) that are characterized by hydroxyl group(s) linked to aromatic cycle(s); often involved in plant–insect interactions.
Phytotoxin: a toxic compound produced by a plant; synonymous here with 'plant toxin' and 'plant allelochemical'.
Phytophagous insect: an insect feeding on plant organs; synonymous here with 'herbivorous insect'.
Resistance cost: resistant individuals have lower fitness levels than do sensitive individuals in the absence of the xenobiotic, thus implying a cost of resistance.
Xenobiotic: a chemical that is found in an organism but which is not normally produced or expected to be present; can also cover substances that are present in unusually high concentrations. In insects, plant-defence chemicals, pesticides, drugs and pollutants are considered to be xenobiotics.

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Table 1. Plant allelochemicals and associated resistance mechanisms in insects

Allelochemical type	Target (and mechanisms of effect)	Resistance mechanisms	Species	Refs
Alkaloids	Neuroreceptors (inhibition); ion channels (antagonist); nucleic acids (disruption of DNA synthesis); feeding (deterrent owing to bitterness); enzymes (inhibition)	Modification of nicotine synthesis by salivary glucose oxidase	*Helicoverpa zea* (Lepidoptera)	[14]
Cardenolides	Nervous system (depressing activity); Na^+, K^+-ATPase (specific inhibitor)	Canal trenching behaviour	*Danaus plexippus* (Lepidoptera)	[11]
		Target-site mutation	*Chrysochus* sp. (Coleoptera)	[56]
Cyanogenic glycosides	Electron transport (inhibition of mitochondrial cytochrome oxidase)	Ingestion avoidance; sequestration and detoxification	*Schistocerca americana* (Orthoptera); *Hypera brunneipennis* (Coleoptera) *Zygaena* sp. (Lepidoptera); *Clossiana euphrosyne* (Lepidoptera) *Heliconius sara* (Lepidoptera)	[9]
Glucosinolates	Respiration (inhibition)	Detoxification by GSTs	*Myzus persicae* (Hemiptera)	[38]
		Detoxification by a glucosinolate sulfatase	*Plutella xylostella* (Lepidoptera)	[49]
		Formation of nitriles instead of isothiocyanate	*Pieris rapae* (Lepidoptera)	[50]
		Detoxification by P450s	*Drosophila melanogaster* (Diptera)	[33]
		Detoxification by N-oxidation and sequestration	*Estigmene acrea* (Lepidoptera); *Tyria jacobaeae* (Lepidoptera)	[47,48]
Flavonoids and phenolic acids	Respiration (inhibition); growth (inhibition)	Ingestion avoidance	*Manduca sexta* (Lepidoptera)	[24]
		Decrease of toxin levels in gall tissue	*Pontania* sp. (Hymenoptera)	[16]
		Glycosylation by UDP – glycosyl–transferase; sequestration and/or excretion	*Bombyx mori* (Lepidoptera)	[45]
Iridoid glycosides	Feeding (deterrent owing to bitterness); nucleic acids (inhibition of DNA polymerase); proteins (denaturant and croos-linking activities)	Sequestration	*Longitarsus* sp. (Coleoptera)	[17]
Coumarins and furanocoumarins	Nucleic acids (photoactive DNA bonding);	Detoxification by P450s	*Papilio polyxenes* (Lepidoptera)	[63]
	Pro-oxidant activity	Detoxification by GSTs	*Depressaria pastinacella* (Lepidoptera); *Spodoptera frugiperda* (Lepidoptera)	[64,65]
Protease inhibitors	Digestive system (inhibition of protease)	Overexpression of insensitive protease	*Callosobruchus maculatus* (Coleoptera)	[46]
Terpenoids	Nervous system (inhibition of acetyl-choline esterase); feeding (deterrent owing to physical barrier and bitterness); growth and development inhibitor (pheromone analog)	Repression of genes involved in biosynthetic pathways	*Spodoptera exigua* (Lepidoptera)	[15]
Tannins	Feeding (complexation of salivary and gut proteins); pro-oxidant activity	Synthesis of anti-oxidant compounds	*Orgyia leucostigma* (Lepidoptera)	[66]

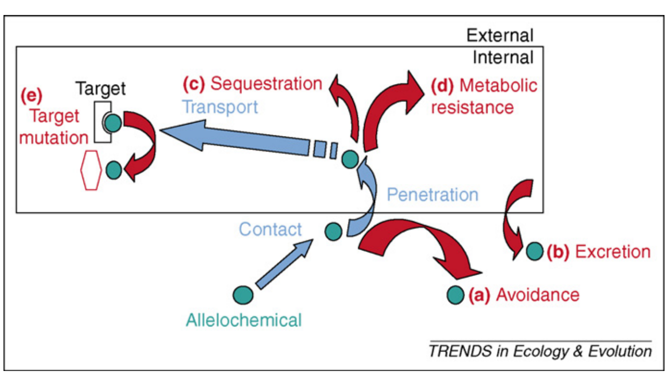

Figure 1. Main steps of plant allelochemical–insect interactions (blue arrows) and resistance mechanisms (red arrows). The first mechanism is avoidance **(a)** of the allelochemical (green circles), which can be genetically determined or acquired by experience after previous contact with the toxic food. Contact avoidance can involve a particular behaviour (e.g. vein cutting) or early deactivation of the allelochemical (e.g. mediated by insect oral secretions). After ingestion, the allelochemical can be readily excreted **(b)**, sequestered **(c)**, or metabolized **(d)** before excretion. Detoxification metabolism occurs in two phases, the first involving oxidation, hydrolysis and/or reduction, the second involving conjugation. These processes involve a variety of enzymes that can be induced after contact with the allelochemical. Finally, mutation in the target of the allelochemical **(e)** can reduce or eliminate its deleterious effects.

and metabolic), and these traits are frequently inducible rather than constitutive. Three main factors influence the costs associated with each resistance trait: the distribution of the toxin in the landscape, the specificity of the plant–insect association and the composition of the local community. Because the pattern of selection pressures occurring in natural environments and insecticide-treated areas differs, cross-resistance between plant chemicals and insecticides can be predicted to be restricted to particular resistance mechanisms and ecological conditions (e.g. metabolic resistance to chemically related plant allelochemicals and insecticides).

Resistance by avoidance of plant toxins

Plant selection and feeding behaviour

Insects can avoid eating toxic plants as soon as they are able to detect them visually, olfactorily or via contact [4]. Avoidance mechanisms can be genetically determined or produced by a learning process. In many cases, genetically determined oviposition behaviour prevents females from laying eggs on unsuitable plants [5]. However, larval performance is not always correlated with oviposition preference and larvae might have to move to select a suitable host plant [6]. Insects can also escape to a toxin-free refuge by feeding selectively on plant organs that do not produce the toxin (i.e. spatial shift or niche restriction), or by exploiting the plant at a stage when the toxin is absent or present at lower levels (i.e. phenological shift) [7].

Several recent studies provide mechanistic insights into the processes involved in food selection by phytophagous insects [4,8]. Bitter foods are often avoided by insects; however, although the molecular bitterness-signaling pathways are highly diversified, they are not always specific to each ingested compound. For example, tobacco hornworm *Manduca sexta* caterpillars cannot discriminate among two different bitter-tasting compounds, a non-toxic phenolic compound (salicin) and a toxic alkaloid (caffeine), because the same bitterness-signalling pathway is activated by both compounds [8]. Similarly, grasshoppers and weevils are deterred by bitter-tasting cyanogenic glucosides that are in concentrations below the toxicity threshold [9]. Therefore, bitterness is not always an honest signal of toxicity and the similarity of pathways activated by toxic and non-toxic allelochemicals might restrict the range of host plants used by an insect, increasing the evolutionary cost of avoidance.

The ecological context can also modify insect feeding behaviour. Solitarious and gregarious-phase *Schistocerca gregaria* locusts respond differently to hyoscyamine, a bitter-tasting plant alkaloid that protects against predators. Solitarious-phase locusts are deterred by the taste of this compound, whereas gregarious-phase locusts accept and even prefer food that contains it. The different feeding behaviour reflects different anti-predator strategies, which also includes differences in coloration and aggregation [10]. In this case, plant toxin ingestion is only beneficial for gregarious individuals that became distasteful, because the learning process of prey avoidance by predators is enhanced when prey are aggregated and coloured (aposematism).

Manipulation of plant chemical defences

Insects can also deactivate the chemical defences of the host plant before feeding. Insects that feed on plants with secretory canals often cut trenches across leaves to depressurize the canals and eliminate toxic exudation at their feeding site [11,12] (Box 1). This time-consuming behaviour was shown to be induced in the lepidopteran polyphagous plusiines by allelochemicals such as lactucin from lettuce latex, myristicin from parsley oil and lobeline from cardinal flowers, but not by several other host-plant toxins also acting as feeding deterrents [11,13]. Therefore, different plant compounds can induce similar or specific insect behavioural responses (e.g. vein cutting and feeding avoidance).

Another way to avoid contact with the toxin is by suppressing plant defences. The induction of plant defences by chewing insects is well documented, and usually involves salivary elicitors. Oral secretions of several caterpillar species have recently been shown to suppress or reduce host-plant defences. The principal component of the tobacco earworm *Helicoverpa zea* saliva is a glucose oxidase that decreases the level of nicotine induced in leaves from tobacco *Nicotiana tabacum* [14]. The same enzyme in the saliva of the beet armyworm *Spodoptera exigua* decreases the transcript level of key regulatory genes involved in the early steps of the defence pathways of the *Medicago truncatula* plant [15]. Another

Box 1. The monarch butterfly: an example of multiple resistance mechanisms

The caterpillar of the monarch butterfly *Danaus plexippus* (Figure Ia) feeds exclusively on milkweeds (*Asclepias* sp) and is an example of a specialist using diverse strategies to overcome host-plant defences. Milkweed latex contains highly toxic cardenolides that vary in amount and type within and among *Asclepias* species, within various plant parts, and over time; other substances are also present, such as cysteine proteases, terpenoids, pregnane glucosides, alkaloids, cyclitols and flavonol glucosides, which are all stored in pressurized latex canals [67]. Late-instar larvae deactivate the defence of milkweeds by cutting veins before feeding [11]. Neither cardenolides nor latex adhesives trigger vein-cutting behaviour, suggesting that other plant compounds might be involved in this behaviour.

Monarch larvae sequester milkweed cardenolides, which are retained by the adults as a defence against predators [19]. This highly efficient chemical protection, together with the aposematic colouration of larvae and adults (Figure I; reproduced with permission from David Dussourd), form the anti-predator strategy of the monarch butterfly [18]. Other milkweed cardenolides, such as uscharidin, are not sequestered but are instead metabolized by aldehyde reductase [68]. Finally, a single amino-acid substitution in the Na^+, K^+-ATPase of the butterfly confers insensitivity to one of the host-plant cardenolides, ouabain [55].

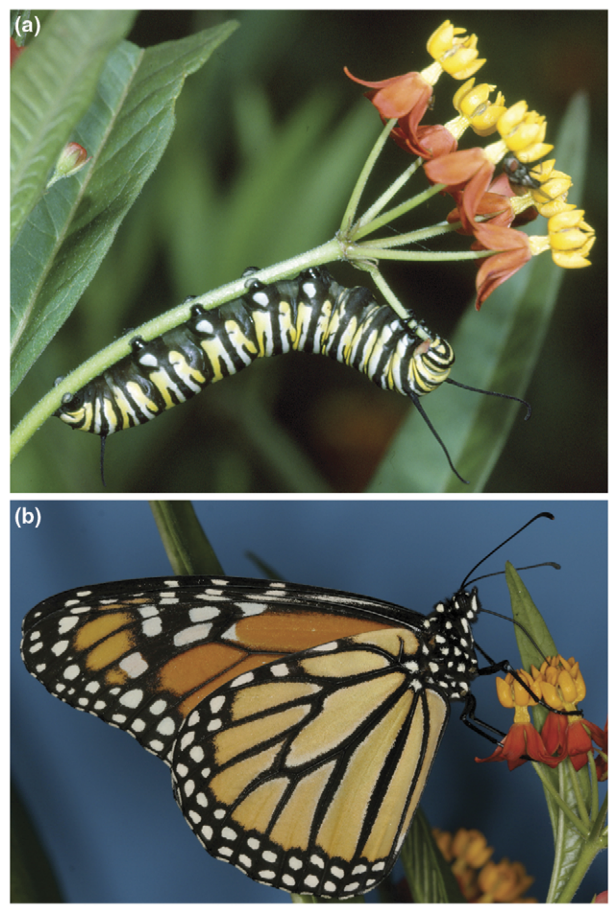

Figure I.

example of manipulation of host-plant chemistry is found in gall-inducing insects such as sawflies, which can decrease the level of toxic phenolic compounds inside the gall where larvae develop, with a striking convergence in the chemical properties of galls induced on different host-plant species, favouring frequent host shifts in this group of insects [16].

Excretion, sequestration and further use of plant toxins

In many insects, a large proportion of the accumulated toxic plant compounds can be excreted, or lost with exuvia during the moult [9]. Plant compounds can also be sequestered [17] and subsequently used as a defensive substance against predators or pathogens [18] as pigments for adult coloration or as pheromones [19] (Box 1). Plant chemicals can also be used to protect against UV light and photo-activated phytotoxins, such as furanocoumarins [20].

Sequestration of compounds necessitates selective transport and storage capabilities that prevent the toxin interfering with the physiological processes of the insect. Recent studies in leaf beetles have shed light on the molecular basis of the sequestration of plant compounds [21]. Despite an apparent complexity of the processes involved in the transport and sequestration of many plant compounds in recently evolved leaf beetle species, the same enzymes with minor modifications are used in the most basal species that produce chemical defences *de novo*. Therefore, although the use of plant compounds as anti-predator defences appears to be a spectacular evolutionary innovation, it requires only a few modifications from ancestral processes. The shift from autogenous to host-derived chemical defence is energetically advantageous and has evolved convergently in different leaf beetle lineages [21,22].

Metabolic resistance to plant toxins

The biotransformation of plant toxins is one of the major weapons that insects have evolved in their coevolutionary arms race with plants [23]. To date, metabolic resistance to plant chemicals has been identified not only in herbivorous insects [24], but also in detritivorous insects such as mosquito larvae feeding on plant debris [25]. Metabolic resistance often results from the overproduction of 'detoxification enzymes' that can metabolize xenobiotics. This mechanism is often associated with phenotypic plasticity, as the production of detoxification enzymes is usually induced by the presence of plant xenobiotics in the diet of the insect. However, resistance to phytotoxins might also be the consequence of specific mutations in genes encoding enzymes, enhancing their catalytic activity toward plant toxins [26].

Overproduction of detoxification enzymes

Detoxification enzymes typically include three main super-families: the cytochrome P450 monooxygenases (P450s or *CYPs* for genes), the glutathione S-transferases (GSTs) and the carboxylesterases (COEs). Among these, P450s (Box 2) appear to have a key role in plant–insect interactions and have been intensively studied [27].

Although the study of the adaptation of Papilionidae lepidopterans to furanocoumarins reveals the complete range of adaptive mechanisms involving P450s according to the varying degrees of host-plant specialization (Box 2), other studies have isolated P450s that are related to the detoxification of plant chemicals in various herbivorous species, such as the parsnip webworm *Depressaria pastinacella* [28], *M. sexta* [29] and several *Helicoverpa* earworm species [30]. In *Helicoverpa zea*, this adaptive strategy has evolved further as the insect can use volatile plant signal molecules (i.e. jasmonate and salicylate) that activate herbivory-induced plant defence pathways as a signal to pre-emptively overproduce P450s that can metabolize host-plant toxins [31]. Another example is the adaptation of cactophilic *Drosophila* species from the Sonoran desert to the specific allelochemicals contained in their host plants, where several unrelated P450-encoding genes are induced by different isoquinoline alkaloids [32], suggesting the exploitation of evolutionarily distant P450 enzymes in the adaptation of the various *Drosophila* species to their different host cacti [33]. In detritivorous insects, overproduction of P450s by mosquito larvae appears to enable them to ingest decaying arborescent leaf litter that contains high levels of secondary plant compounds, broadening their habitat range [25,34,35].

The GST superfamily has also been involved in the detoxification of various plant xenobiotics. Although GST enzymes can be involved in substrate sequestration, they usually catalyse the conjugation of glutathione to electrophilic toxic molecules, increasing their solubility and facilitating their elimination by the insect [36]. As in P450s, GST-mediated metabolism is often induced by the ingestion of allelochemicals. The role of GSTs in the resistance to plant chemicals has been studied in numerous crop-feeding lepidopteran species [37] and in insects feeding on deciduous trees [37]. In the aphid *Myzus persicae*, the overproduction of GSTs is probably responsible for the adaptation of the insect to glucosinolates and isothiocyanates contained in its Brassicaceae host plants [38]. This enzymatic induction has also been reported in larvae of the predatory hoverfly *Episyrphus balteatus*, which feed on *Myzus* aphids, suggesting that the accumulation of plant toxins or metabolites in aphids also induces metabolically based resistance mechanisms in their specific predator species [39]. As in P450s, the greater diversification of GST enzymes in generalist than in specialist herbivores is thought to reflect the ability of such herbivores to adapt to a broader range of plant chemicals [38,40].

Carboxylesterases are enzymes that can hydrolyse ester bonds from various substrates with a carboxylic ester. To date, few studies have demonstrated their role in the adaptation of insects to plant chemicals. However, their involvement in the metabolism of other xenobiotics, such as plant-derived insecticides (e.g. pyrethroids), supports their putative involvement in plant toxins degradation [41,42].

Another enzyme family implicated in insect resistance to plant chemicals is the UDP-glycosyltransferases (UGTs) [43], which act as catalysts for the transfer of a glycosyl group from UDP-glucose to a variety of acceptor molecules. To date, only limited information is available about their role in the detoxification of xenobiotics by insects. Metabolism of plant compounds by UGTs has been reported in *M. sexta* [44]; the gene encoding another UGT (*BmUGT1*), which is involved in the degradation of flavonoids and

Box 2. Cytochrome P450 monooxygenases and resistance to plant toxins

Cytochrome P450 monooxygenases (P450s or *CYPs* for encoding genes) are 45–55-kDa heme-thiolate enzymes found in all living organisms and represent one of the largest gene superfamilies [27,69]. 'P450' reflects the typical absorption peak of their Fe^{II}–CO complex at 450 nm. These enzymes can catalyse a range of chemical reactions, but are best known for their monooxygenase reaction, whereby they catalyse the transfer of one atom of molecular oxygen to a substrate while reducing the other to water (Figure I). *CYP* genes are classified into different families (e.g. *CYP6* family) and subfamilies (e.g. *CYP6B* subfamily) according to their amino-acid sequence homology, and have been isolated from various insect species; 50 to >100 different *CYP*s have been found in those species for which genome annotation is available [70–72], although their biological function is often uncertain. Although P450 retrotransposition can occur, gene duplication appears to be the main evolutionary mechanism for P450 diversification, leading to the formation of genomic clusters of related *CYP* genes [70]. *CYP* gene expression is frequently induced and/or repressed by xenobiotics, can vary according to insect developmental stages and sex, and is sometime restricted to specific organs and tissues. Insect P450s appear to be specialized in the metabolism of endogenous substrates (e.g. hormones, pheromones, cuticle hydrocarbons, fatty acids, defensive compounds, etc.) and exogenous compounds (e.g. drugs, insecticides, pollutants, plant compounds, etc.). P450 web links and *CYP* sequences from various insects are available from http://p450.antibes.inra.fr/index.html.

The best-documented example of the role of P450s in plant–insect interactions is the adaptation of Papilionidae lepidopterans to cope with toxic furanocoumarin compounds that are found in their host plants. Inducible furanocoumarin metabolism was found in typical detoxifying organs (e.g. midgut and fat bodies) of the specialist herbivore *Papilio polyxenes* (black swallowtail, Figure II), which feeds exclusively on furanocoumarins-containing plants (e.g. wild parsnip) [63,73]. A multi-allelic gene (*CYP6B1*) was identified that encodes a P450 specifically induced by xanthotoxin, a linear furanocoumarin [74]. Expression of *CYP6B1* in a baculovirus system revealed the ability of its encoded enzyme to metabolize both linear and angular furanocoumarins [75], with greater efficiency for furanocoumarins for which *P. polyxenes* is specifically adapted [76]. Comparison between *CYP6B* genes from the specialist herbivore *P. polyxenes* and more generalist *Papilio* species has revealed a wider spectrum of *CYP6Bs* for generalist species, which are more likely to encounter various furanocoumarins in their diet compared with specialist species [76,77].

Recently, comparison of *CYP6B* upstream regulatory sequences of various *Papilio* species revealed the involvement of conserved transcription-regulatory elements in the regulation of the basal expression of *CYP6B* genes and their induction by furanocoumarins [78,79]. Therefore, the recent divergence of *CYP6B* genes during *Papilio* species specialization has resulted in divergence not only in the coding sequence of those genes, but also in their promoter regions, suggesting that both transcription regulation and changes in catalytic site activity have a role in the adaptation of insects to plant toxins through metabolic resistance mechanisms [80].

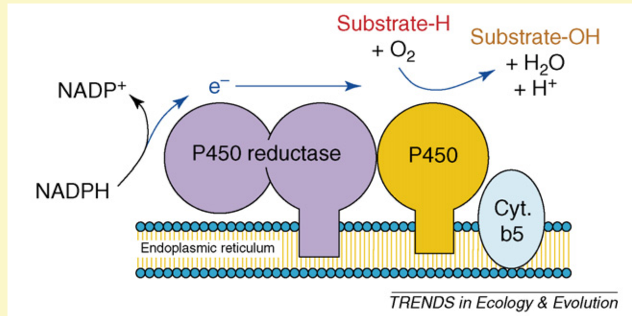

Figure I. An example of a P450 system from the endoplasmic reticulum. In eukaryotes, cytochrome P450 monooxygenases (P450, yellow) are membrane-bound proteins located in either the endoplasmic reticulum or mitochondria. They use NADPH as an electron donor and depend on other redox partners for electron transfer (blue arrows), such as cytochrome P450 reductase (purple) and cytochrome *b*5 (Cyt b5, light blue) in endoplasmic reticulum or ferredoxin and ferredoxin reductase in mitochondria.

Figure II. The larva of the black swallowtail butterfly *Papilio polyxenes* feeding on its host plant. Reproduced with permission from Suzan Ellis and USDA Forest Service Archives (http://www.forestryimages.org).

coumarins, has recently been isolated in the silkworm *Bombyx mori* [45].

A unifying feature of detoxification enzymes is their diversification in insects, making it difficult to characterize the genes involved in resistance to plant toxins. With the increasing availability of full insect genome sequences (e.g. the fruit fly, the malaria and yellow-fever mosquitoes, the honey bee, the silkworm and the red flour beetle), the ever-increasing number of insect expressed sequence tags (ESTs), and recent advances in functional and comparative genomics, it is now possible to study the role of detoxifying genes in insect resistance to plant toxins.

Other metabolic resistance mechanisms

Despite the major role that detoxification enzymes have in insect resistance to plant toxins, other metabolic resistance mechanisms have also been identified in various plant–insect interactions.

Larvae of the cowpea bruchid *Callosobruchus maculatus* (Coleoptera) fed on a diet containing the soybean cysteine protease inhibitor soyacystatin N (scN) activate an array of counterdefence genes to adapt to this toxin. Using micro-array technology, Monn *et al.* [46] suggested that the over-expression of cathepsin-like cysteine proteases has a key role in the adaptation of this insect to ingested scN by saturating the protease inhibitor molecules.

When the polyphagous arctiid moth *Estigmene acrea* (Lepidoptera) feeds on species of Asteraceae, it not only sequesters pyrrolizidine alkaloids (PAs) contained in those plants, but also detoxifies them by N-oxidation catalysed by a specific flavin-dependent monooxygenase [47]. The *in vitro* expression of a similar N-oxygenase from the arctiid moth *Tyria jacobaeae* confirmed its high metabolic activity toward PAs, which enables the insect to feed on plants containing toxic PAs and to accumulate PA metabolites as a predator deterrent [48].

Another interesting example is the response of the diamondback moth *Plutella xylostella* to the defence system of its crucifer host plants, the 'mustard oil bomb'. This system relies on the co-secretion of glucosinolates and a specific enzyme (myrosinase), each stored in separate cell compartments. Following tissue damage, glucosinolates are hydrolysed by myrosinase into highly toxic products, such as isothiocyanate. The diamondback moth has developed an original adaptive strategy based on the modification of ingested plant glucosinolates by a sulfatase gut enzyme, preventing their hydrolysis by plant myrosinase, thus disarming the 'mustard oil bomb' [49]. The cabbage white butterfly *Pieris rapae* has developed a different adaptive mechanism by redirecting toxic isothiocyanate formation toward non-toxic nitrile formation with a specific gut protein [50].

Association with symbiotic microorganisms

Many insects live in close association with microorganisms (e.g. plant sap-sucking insects with bacterial endosymbionts) [51]. Given the many enzymatic activities known to occur in bacteria and fungi, their role in detoxifying secondary plant compounds has been suspected but not yet clearly demonstrated [52]. Further research will involve evaluating the role of endosymbionts in the detoxification of plant toxins.

Metabolic resistance plasticity and host-plant specialization

Although evolutionary factors leading to the adaptation of insects to plant toxins through metabolic resistance mechanisms are not yet fully understood, the induction of detoxification enzymes might confer an adaptive plasticity to insects that enables them to optimize their fitness in the presence of varying levels of toxins. Furthermore, a mutation leading to an increased enzymatic affinity toward a particular xenobiotic is more likely to be retained by selection if it occurs in an inducible gene that overproduces the enzyme, rather than in a constitutive gene, because it will readily confer a higher resistance to its borer in presence of the toxin [53]. Therefore, the induction of detoxification genes by plant toxins could be considered as a first step toward further selection of more specific mutations that lead to specialization of the insect if suitable ecological conditions are satisfied (i.e. high probability of encountering the toxin). Indeed, mutations of detoxification enzymes leading to increased activity toward particular phytotoxins have been found in highly specialized insects (e.g. *Depressaria pastinacella*) [54].

Mutation in the target site of the phytotoxin

Although mutations conferring constitutive resistance to insecticides have been found in various insect species [1], few studies have identified mutations leading to insect resistance to specific host-plant chemicals. The only well-documented example is from two unrelated lineages, the specialist monarch butterfly and two leaf beetle (*Chrysochus*) species, which are all resistant to ouabain, a toxic cardenolide found in their milkweed host plants (Box 1). In this case, a single amino-acid substitution in the target site of ouabain (the Na^+, K^+-ATPase) is responsible for resistance [55], and a recent molecular phylogenetic analysis of *Chrysochus* showed that the evolutionary host-plant switch to cardenolide-containing plants coincided with the same amino-acid substitution in the ouabain binding site [56].

Why are the target-site mutations that are selected when using insecticides (often chemically similar to plant chemicals) so rarely observed in insect populations that are resistant to plant toxins? It is possible that, because the modified target site does not function as well as the original site, the cost of such mutations is too high for them to spread in natural populations, and adaptive mechanisms such as behavioural avoidance and metabolic resistance are more advantageous at the evolutionary scale. In support of this hypothesis, insect populations treated for several decades with the same insecticide have been shown to evolve less costly resistance mechanisms [57].

Evolutionary insights

Several mechanisms are usually combined in insects to cope with plant allelochemicals. For example, behavioural avoidance of a phytotoxin is often associated with the ability to metabolize it, and sequestration is often associated with insect insensitivity to the toxin (Box 1; Figure 1). Despite this general trend, genetic linkage between these different resistance traits has not yet been demonstrated, and their co-occurrence might result from ecological rather than from genetic constraints. If the insensitivity to the sequestered toxin as a prerequisite to the evolution of sequestration abilities is not surprising, the co-occurrence of toxin avoidance and metabolic degradation appears paradoxical. Why do insects simultaneously invest in both behavioural and metabolic resistances, given that avoiding the phytotoxin is unnecessary if it is destroyed anyway? As suggested by a theoretical model [58], reinforcement of metabolic resistance to host defences by avoidance might be optimal if the fitness costs of the resistance traits are accelerating and the probability of encountering defended hosts is low.

Costs and benefits of resistance to plant toxins

The cost–benefit outcome of the different resistance traits depends on the spatial distribution of the toxin in the landscape. Plants present variable levels of toxicity not only among, but also within species. The level of toxicity expressed by a plant depends on its genotype as well as on its resource allocation to defence, which varies with its phenological stage and its biotic and abiotic environment. Behavioural resistance through avoidance of the more toxic plants or organs involves time costs (time spent for searching suitable hosts, or deactivating the host-plant defence), which increase with the frequency of defended versus undefended plants. Similarly, induced metabolic resistance involves energetic costs that increase with the probability of encountering a defended plant and with its toxicity level. Therefore, both behavioural and metabolic resistance traits involve increasing frequency-dependent costs. Sequestration also involves energetic costs (selective transport), although this can be overcome by ecological benefits (e.g. anti-predator defence or UV protection). The net outcome of maintaining this trait therefore depends strongly on local ecological constraints. Finally, target-site mutation might involve a cost in terms of a decrease in efficiency of the mutated insect function. Contrary to all the other resistance traits, this cost is fixed, depends on the function of the target gene and does not increase with the probability of encountering defended plants.

A second general feature of insect resistance traits is their phenotypic plasticity. Overproduction of detoxification enzymes and avoidance behaviour are usually induced by the presence of phytotoxins, whereas constitutive mutations in the target site of the phytotoxin are rare and restricted to a few specialists. Interestingly, some specialists maintain several resistance mechanisms simultaneously (Box 1) which might enable them to handle the diverse toxins produced by their host plant. Finally, maintaining several induced resistance mechanisms can reduce the long-term evolutionary cost of resistance and the risk of extinction by enabling host-plant shifts in both generalists and specialists [22].

Plant toxins, insecticides and cross-resistance mechanisms

Plant toxins are heterogeneously distributed in the landscape, whereas insecticides are more homogeneously distributed throughout the treated area. Moreover, plants frequently produce several toxins simultaneously, whereas insecticide spraying usually involves a single molecule frequently acting on a specific target. This results in the selection of both behavioural and metabolic resistance traits in polyphagous insects, with the probability of selection of additional resistance traits such as sequestration capability and more specific mutations increasing with the probability of encountering a particular toxin (i.e. insect specialization, Figure 2).

In insecticide-treated areas, the probability of encountering a specific toxin is high, and specific mutations in detoxifying enzymes or in the target-site of the insecticide will be readily selected [1]. Therefore, the probability of encountering a particular toxin appears to be central in evolving particular resistance traits (Figure 2). In addition, the whole local community will influence the relative strength of selective pressures owing to xenobiotics. In insecticide-treated areas, the strong selection pressure from insecticides might overcome the importance of other selection pressures; by contrast, plant toxicity is often more diffuse and can give more importance to other selection pressures.

Despite these differences, cross-resistance between plant allelochemicals and chemical insecticides is an acknowledged phenomenon [27]. However, only a few studies have

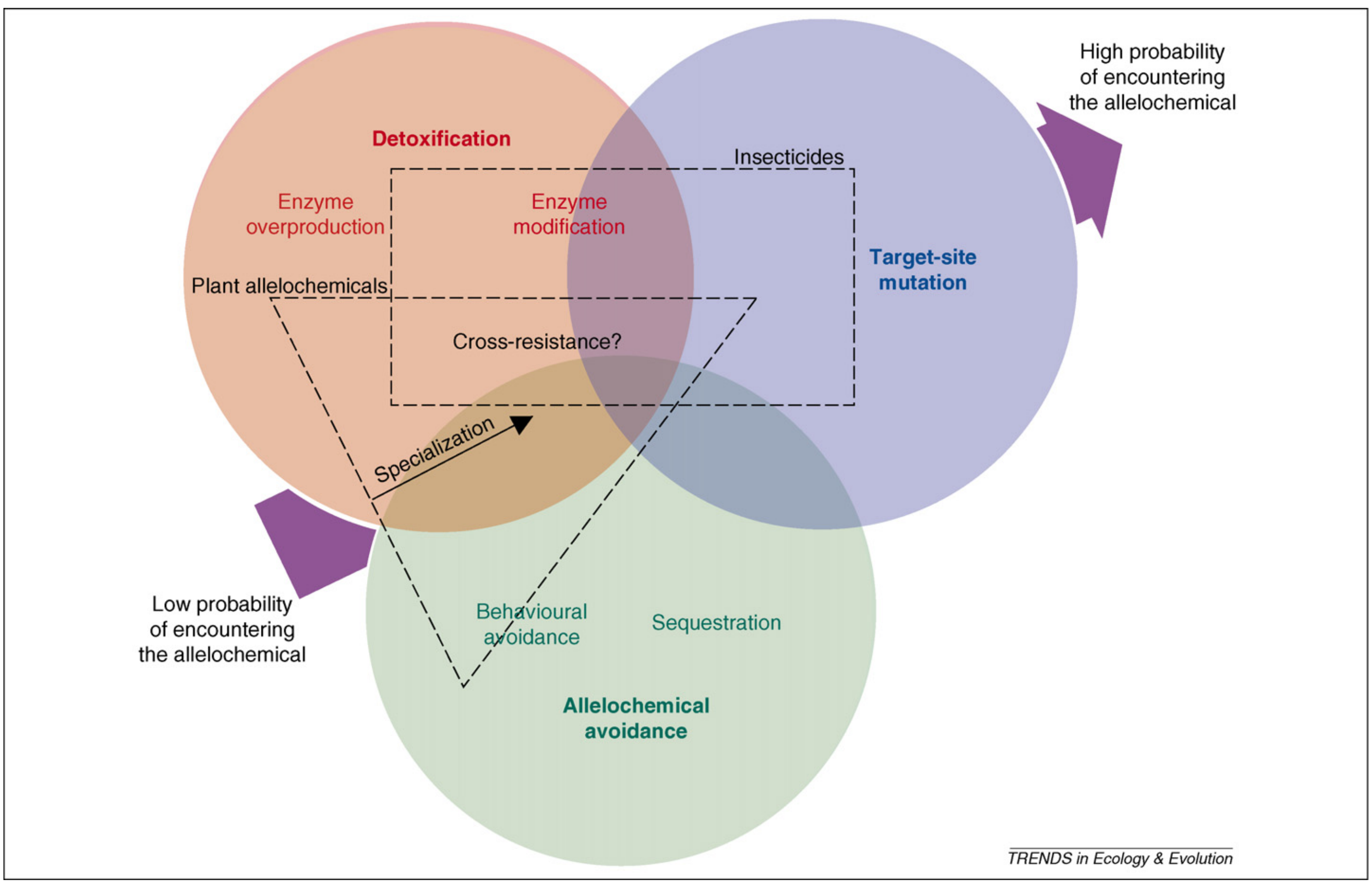

Figure 2. The adaptive mechanisms involved in the resistance of insects to plant allelochemicals (dashed triangle) and insecticides (dashed rectangle). Resistance owing to target-site mutation (blue circle) is often encountered in insects that are resistant to insecticides, whereas it is rarely observed in insects exploiting toxic plants, even in specialist herbivores. Detoxification-based resistance mechanisms, such as enzyme overproduction and modification (red circle), are found in both situations and might lead to cross-resistance. Avoidance of allelochemicals (green circle) can be behavioural, as found mainly in insects that are resistant to plant chemicals, or can involve sequestration mechanisms. Among the factors driving the type of resistance evolved in insects, the increasing probability of encountering a specific allelochemical (purple arrow) might favour both enzyme modifications that result in a more efficient metabolism of the xenobiotic and target site mutations, leading to higher tolerance and, thus, specialization of the insect on that particular host plant.

shown increased resistance to insecticides in insects fed plant allelochemicals. To date, most identified cross-resistance mechanisms between plant toxins and insecticides involve metabolic resistance through detoxification enzymes. This is not surprising as a single detoxification enzyme might metabolize different substrates and catalyse different biochemical reactions. For example, after exposure of corn earworm *H. zea* larvae to xanthotoxin, survivors and their offspring displayed higher tolerance to the pyrethroid insecticide α-cypermethrin, suggesting that this increased resistance is heritable [59]. Fall armyworm *S. frugiperda* larvae fed on corn were shown to become less susceptible to various insecticides than were larvae fed on soybean, owing to enhanced monooxygenases activity. Similarly, larvae from this species fed on cowpeas, a potent inducer of GSTs, were twice as tolerant to organophosphorus insecticides than were those fed on soybean [3].

By contrast, exposure to particular plant chemicals can repress the expression of detoxification enzymes involved in insecticide resistance. For example, several plant phenolic compounds were shown to inhibit GSTs in the fall armyworm larvae and could, therefore, enhance insecticide efficiency [60]. Finally, it cannot be excluded that an enzyme conferring resistance to a phytotoxin can enhance the toxicity of an insecticide and vice versa.

The striking complexity of the repression–induction patterns and substrate specificities of detoxification enzymes has so far represented a major difficulty in the understanding of cross-resistance mechanisms. The recent genome sequencing of many insect species, together with the development of functional genomics techniques (e.g. gene expression microarrays) and high-throughput genotyping technologies, represents a valuable opportunity to study cross-resistance mechanisms between phytotoxins, insecticides and other xenobiotics, such as pollutants [61,62].

Conclusions and further research directions

Over 400 million years of coevolution with plants, phytophagous insects have developed diverse resistance mechanisms to cope with plant chemical defences. Because insects face a geographical mosaic of chemical environments, from non-toxic to highly toxic plants, the costs associated with resistance traits vary with the probability of encountering a toxin. Moreover, other selection pressures, such as the presence or absence of competitors and predators, can also influence the costs and selection of particular resistance traits. Thus, the complexity of the local community composition is a key factor in maintaining the diversity of adaptive mechanisms to plant toxins. These mechanisms are more plastic and complex compared with those involved in resistance to insecticides, perhaps because environments in which insecticides are heavily used also tend to have communities of low diversity and complexity. However, because some detoxification enzymes are involved in plant toxins and insecticides metabolism, cross-resistance mechanisms can be predicted to be observed under specific environmental conditions (Figure 2). Deciphering the impact of allelochemicals in cross-resistance mechanisms with insecticides at a local scale, and comparing the molecular and evolutionary mechanisms of resistance to phytotoxins and insecticides, represents promising areas of research for developing long-term sustainable insect control strategies.

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References

1 ffrench-Constant, R.H. *et al.* (2004) The genetics and genomics of insecticide resistance. *Trends Genet.* 20, 163–170
2 Ferre, J. and Van Rie, J. (2002) Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. *Annu. Rev. Entomol.* 47, 501–533
3 Yu, S.J. and Ing, R.T. (1984) Microsomal biphenyl hydroxylase of fall armyworm larvae and its induction by allelochemicals and host plants. *Comp. Biochem. Physiol. C* 78, 145–152
4 Chapman, R.F. (2003) Contact chemoreception in feeding by phytophagous insects. *Annu. Rev. Entomol.* 48, 455–484
5 Fox, C.W. *et al.* (2004) Genetic architecture of population differences in oviposition behaviour of the seed beetle *Callosobruchus maculatus*. *J. Evol. Biol.* 17, 1141–1151
6 Cotter, S.C. and Edwards, O.R. (2006) Quantitative genetics of preference and performance on chickpeas in the noctuid moth, *Helicoverpa armigera*. *Heredity* 96, 396–402
7 Nealis, V.G. and Nault, J.R. (2005) Seasonal changes in foliar terpenes indicate suitability of Douglas-fir buds for western spruce budworm. *J. Chem. Ecol.* 31, 683–696
8 Glendinning, J.I. *et al.* (2002) Contribution of different taste cells and signaling pathways to the discrimination of "bitter" taste stimuli by an insect. *J. Neurosci.* 22, 7281–7287
9 Zagrobelny, M. *et al.* (2004) Cyanogenic glucosides and plant–insect interactions. *Phytochemistry* 65, 293–306
10 Despland, E. and Simpson, S.J. (2005) Food choices of solitarious and gregarious locusts reflect cryptic and aposematic antipredator strategies. *Anim. Behav.* 69, 471–479
11 Helmus, M.R. and Dussourd, D.E. (2005) Glues or poisons: which triggers vein cutting by monarch caterpillars? *Chemoecology* 15, 45–49
12 Becerra, J.X. (2003) Synchronous coadaptation in an ancient case of herbivory. *Proc. Natl. Acad. Sci. U. S. A.* 100, 12804–12807
13 Dussourd, D.E. (2003) Chemical stimulants of leaf-trenching by cabbage loopers: Natural products, neurotransmitters, insecticides, and drugs. *J. Chem. Ecol.* 29, 2023–2047
14 Musser, R.O. *et al.* (2002) Herbivory: Caterpillar saliva beats plant defences - a new weapon emerges in the evolutionary arms race between plants and herbivores. *Nature* 416, 599–600
15 Bede, J.C. *et al.* (2006) Caterpillar herbivory and salivary enzymes decrease transcript levels of *Medicago truncatula* genes encoding early enzymes in terpenoid biosynthesis. *Plant Mol. Biol.* 60, 519–531
16 Nyman, T. and Julkunen-Tiitto, R. (2000) Manipulation of the phenolic chemistry of willows by gall-inducing sawflies. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13184–13187
17 Willinger, G. and Dobler, S. (2001) Selective sequestration of iridoid glycosides from their host plants in *Longitarsus* flea beetles. *Biochem. Syst. Ecol.* 29, 335–346
18 Ode, P.J. (2006) Plant chemistry and natural enemy fitness: effects on herbivore and natural enemy interactions. *Annu. Rev. Entomol.* 51, 163–185
19 Nishida, R. (2002) Sequestration of defensive substances from plants by Lepidoptera. *Annu. Rev. Entomol.* 47, 57–92
20 Carroll, M. *et al.* (1997) Behavioral effects of carotenoid sequestration by the parsnip webworm, *Depressaria pastinacella*. *J. Chem. Ecol.* 23, 2707–2719
21 Kuhn, J. *et al.* (2004) Selective transport systems mediate sequestration of plant glucosides in leaf beetles: a molecular basis for adaptation and evolution. *Proc. Natl. Acad. Sci. U. S. A.* 101, 13808–13813
22 Termonia, A. *et al.* (2001) Feeding specialization and host-derived chemical defense in Chrysomeline leaf beetles did not lead to an evolutionary dead end. *Proc. Natl. Acad. Sci. U. S. A.* 98, 3909–3914
23 Berenbaum, M.R. (2002) Postgenomic chemical ecology: from genetic code to ecological interactions. *J. Chem. Ecol.* 28, 873–895

24 Glendinning, J.I. (2002) How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomol. Exp. App.* 104, 15–25
25 Meyran, J.C. *et al.* (2002) The biochemical basis of dietary polyphenols detoxification by aquatic detritivorous Arthropoda. *Rec. Res. Dev. Anal. Biochem.* 2, 185–199
26 Wen, Z. *et al.* (2005) Ile115Leu mutation in the SRS1 region of an insect cytochrome P450 (CYP6B1) compromises substrate turnover via changes in a predicted product release channel. *Protein Eng. Des. Sel.* 18, 191–199
27 Feyereisen, R. (2005) Insect cytochrome P450. In *Comprehensive Molecular Insect Science* (Gilbert, L.I. *et al.*, eds), pp. 1–77, Elsevier
28 Cianfrogna, J.A. *et al.* (2002) Dietary and developmental influences on induced detoxification in an oligophage. *J. Chem. Ecol.* 28, 1349–1364
29 Stevens, J.L. *et al.* (2000) Inducible P450s of the CYP9 family from larval *Manduca sexta* midgut. *Insect Biochem. Mol. Biol.* 30, 559–568
30 Li, X. *et al.* (2002) Plant allelochemicals differentially regulate *Helicoverpa zea* cytochrome P450 genes. *Insect Mol. Biol.* 11, 343–351
31 Li, X. *et al.* (2002) Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature* 419, 712–715
32 Fogleman, J.C. *et al.* (1998) The molecular basis of adaptation in *Drosophila* - The role of cytochrome P450s. *Evol. Biol.* 30, 15–77
33 Fogleman, J.C. (2000) Response of *Drosophila melanogaster* to selection for P450-mediated resistance to isoquinoline alkaloids. *Chem. Biol. Interact.* 125, 93–105
34 David, J.P. *et al.* (2002) Larvicidal properties of decomposed leaf litter in the subalpine mosquito breeding sites. *Environ. Toxicol. Chem.* 21, 62–66
35 David, J.P. *et al.* (2006) Involvement of cytochrome P450 monooxygenases in the response of mosquito larvae to dietary plant xenobiotics. *Insect Biochem. Mol. Biol.* 36, 410–420
36 Enayati, A.A. *et al.* (2005) Insect glutathione transferases and insecticide resistance. *Insect Mol. Biol.* 14, 3–8
37 Yu, S.J. (1996) Insect glutathione *S*-transferases. *Zool. Stud.* 35, 9–19
38 Francis, F. *et al.* (2005) Glutathione S-transferases in the adaptation to plant secondary metabolites in the *Myzus persicae* aphid. *Arch. Insect Biochem. Physiol.* 58, 166–174
39 Vanhaelen, N. *et al.* (2001) Hoverfly glutathione S-Transferases and effect of Brassicaceae secondary metabolites. *Pestic. Biochem. Physiol.* 71, 170–177
40 Francis, F. *et al.* (2001) Effects of allelochemicals from first (Brassicaceae) and second (*Myzus persicae* and *Brevicoryne brassicae*) trophic levels on *Adalia bipunctata*. *J. Chem. Ecol.* 27, 243–256
41 Yang, Z. *et al.* (2005) Molecular dynamics of detoxification and toxin-tolerance genes in brown planthopper (*Nilaparvata lugens* Stal., Homoptera: Delphacidae) feeding on resistant rice plants. *Arch. Insect Biochem. Physiol.* 59, 59–66
42 Usmani, K.A. and Knowles, C.O. (2001) DEF sensitive esterases in homogenates of larval and adult *Helicoverpa zea, Spodoptera frugiperda*, and *Agrotis ipsilon* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 94, 884–891
43 Mackenzie, P.I. *et al.* (2005) Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet. Genomics* 15, 677–685
44 Ahmad, S.A. and Hopkins, T.L. (1993) *B*-glycosylation of plant phenolics by phenol *B*-glucosyltransferase in larval tissues of the tobacco hornworm, *Manduca sexta* (L.). *Insect Biochem. Mol. Biol.* 23, 581–589
45 Luque, T. *et al.* (2002) Characterization of a novel silkworm (*Bombyx mori*) phenol UDP-glucosyltransferase. *Eur. J. Biochem.* 269, 819–825
46 Moon, J. *et al.* (2004) Transcriptional regulation in cowpea bruchid guts during adaptation to a plant defence protease inhibitor. *Insect Mol. Biol.* 13, 283–291
47 Hartmann, T. *et al.* (2005) Specific recognition, detoxification and metabolism of pyrrolizidine alkaloids by the polyphagous arctiid *Estigmene acrea*. *Insect Biochem. Mol. Biol.* 35, 391–411
48 Naumann, C. *et al.* (2002) Evolutionary recruitment of a flavin-dependent monooxygenase for the detoxification of host plant-acquired pyrrolizidine alkaloids in the alkaloid-defended arctiid moth *Tyria jacobaeae*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 6085–6090
49 Ratzka, A. *et al.* (2002) Disarming the mustard oil bomb. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11223–11228
50 Wittstock, U. *et al.* (2004) Successful herbivore attack due to metabolic diversion of a plant chemical defense. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4859–4864
51 Baumann, P. (2005) Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Microbiol.* 59, 155–189
52 Dowd, P.F. (1992) Insect fungal symbionts - a promising source of detoxifying enzymes. *J. Ind. Microbiol.* 9, 149–161
53 Le Goff, G. *et al.* (2006) Xenobiotic response in *Drosophila melanogaster*: sex dependence of P450 and GST gene induction. *Insect Biochem. Mol. Biol.* 36, 674–682
54 Mao, W. *et al.* (2006) Remarkable substrate-specificity of CYP6AB3 in *Depressaria pastinacella*, a highly specialized caterpillar. *Insect Mol. Biol.* 15, 169–179
55 Holzinger, F. and Wink, M. (1996) Mediation of cardiac glycoside insensitivity in the Monarch butterfly (*Danaus plexippus*): Role of an amino acid substitution in the ouabain binding site of Na^+, K^+-ATPase. *J. Chem. Ecol.* 22, 1921–1937
56 Labeyrie, E. and Dobler, S. (2004) Molecular adaptation of *Chrysochus* leaf beetles to toxic compounds in their food plants. *Mol. Biol. Evol.* 21, 218–221
57 Raymond, M. *et al.* (2001) Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation? *Genetica* 112-113, 287–296
58 Vacher, C. *et al.* (2005) Avoid, attack or do both? Behavioral and physiological adaptations in natural enemies faced with novel hosts. *BMC Evol. Biol.* 5, 60
59 Li, X. *et al.* (2000) Cross-resistance to alpha-cypermethrin after xanthotoxin ingestion in *Helicoverpa zea* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 93, 18–25
60 Yu, S.J. and Abo-Elghar, G.E. (2000) Allelochemicals as inhibitors of glutathione S-transferases in the fall armyworm. *Pestic. Biochem. Physiol.* 68, 173–183
61 Pedra, J.H. *et al.* (2004) Genome-wide transcription profile of field- and laboratory-selected dichlorodiphenyltrichloroethane (DDT)-resistant *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 101, 7034–7039
62 David, J.P. *et al.* (2005) The *Anopheles gambiae* detoxification chip: a highly specific microarray to study metabolic-based insecticide resistance in malaria vectors. *Proc. Natl. Acad. Sci. U. S. A.* 102, 4080–4084
63 Petersen, R.A. *et al.* (2001) Expression of CYP6B1 and CYP6B3 cytochrome P450 monooxygenases and furanocoumarin metabolism in different tissues of *Papilio polyxenes* (Lepidoptera: Papilionidae). *Insect Biochem. Mol. Biol.* 31, 679–690
64 Nitao, J.K. *et al.* (2003) Characterization of furanocoumarin metabolites in parsnip webworm, *Depressaria pastinacella*. *J. Chem. Ecol.* 29, 671–682
65 Yu, S.J. (2002) Biochemical characteristics of microsomal and cytosolic glutathione *S*-transferases in larvae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). *Pestic. Biochem. Physiol.* 72, 100–110
66 Barbehenn, R.V. *et al.* (2003) Antioxidants in the midgut fluids of a tannin-tolerant and a tannin-sensitive caterpillar: effects of seasonal changes in tree leaves. *J. Chem. Ecol.* 29, 1099–1116
67 Zalucki, M.P. *et al.* (2001) Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. *Ecol. Entomol.* 26, 212–224
68 Marty, M.A. and Krieger, R.I. (1984) Metabolism of uscharidin, a milkweed cardenolide, by tissue-homogenates of monarch butterfly larvae, *Danaus-plexippus* L. *J. Chem. Ecol.* 10, 945–956
69 Werck-Reichhart, D. and Feyereisen, R. (2000) Cytochromes P450: a success story. *Genome Biol.* 1, 1–9
70 Tijet, N. *et al.* (2001) The cytochrome P450 superfamily in *Drosophila melanogaster*: Annotation, intron-exon organization and phylogeny. *Gene* 262, 189–198
71 Ranson, H. *et al.* (2002) Evolution of supergene families associated with insecticide resistance. *Science* 298, 179–181
72 Claudianos, C. *et al.* (2006) A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. *Insect Mol. Biol.* 15, 615–636
73 Harrison, T.L. *et al.* (2001) Developmental variation in cytochrome P450 expression in *Papilio polyxenes* in response to xanthotoxin, a hostplant allelochemical. *Arch. Insect Biochem. Physiol.* 48, 179–189
74 Cohen, M.B. *et al.* (1992) A host-inducible cytochrome P-450 from a host-specific caterpillar: molecular cloning and evolution. *Proc. Natl. Acad. Sci. U. S. A.* 89, 10920–10924

75 Wen, Z. *et al.* (2003) Metabolism of linear and angular furanocoumarins by *Papilio polyxenes* CYP6B1 co-expressed with NADPH cytochrome P450 reductase. *Insect Biochem. Mol. Biol.* 33, 937–947
76 Li, W. *et al.* (2003) Diversification of furanocoumarin-metabolizing cytochrome P450 monooxygenases in two papilionids: specificity and substrate encounter rate. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14593–14598
77 Li, W. *et al.* (2001) Molecular analysis of multiple CYP6B genes from polyphagous *Papilio* species. *Insect Biochem. Mol. Biol.* 31, 999–1011
78 Brown, R.P. *et al.* (2005) Regulation of an insect cytochrome P450 monooxygenase gene (CYP6B1) by aryl hydrocarbon and xanthotoxin response cascades. *Gene* 358, 39–52
79 McDonnell, C.M. *et al.* (2004) Conserved regulatory elements in the promoters of two allelochemical-inducible cytochrome P450 genes differentially regulate transcription. *Insect Biochem. Mol. Biol.* 34, 1129–1139
80 Li, W. *et al.* (2002) CYP6B cytochrome P450 monooxygenases from *Papilio canadensis* and *Papilio glaucus*: potential contributions of sequence divergence to host plant associations. *Insect Mol. Biol.* 11, 543–551

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REVIEW

Can mechanism help explain insect host choice?

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information processing;
odours;
olfaction.

Abstract

Evolutionary theory predicts that herbivorous insects should lay eggs on plants in a way that reflects the suitability of each plant species for larval development. Empirical studies, however, often fail to find any relationship between an adult insect's choice of host–plant and offspring fitness, and in such cases, it is generally assumed that other 'missing' factors (e.g. predation, host–plant abundance, learning and adult feeding sites) must be contributing to overall host suitability. Here, I consider an alternative theory – that a fitness cost inherent in the olfactory mechanism could constrain the evolution of insect host selection. I begin by reviewing current knowledge of odour processing in the insect antennal lobe with the aid of a simple schematic: the aim being to explain the workings of this mechanism to scientists who do not have prior knowledge in this field. I then use the schematic to explore how an insect's perception of host and non-host odours is governed by a set of processing rules, or algorithm. Under the assumptions of this mechanistic view, the perception of every plant odour is interrelated, and seemingly bad host choices can still arise as part of an overall adaptive behavioural strategy. I discuss how an understanding of mechanism can improve the interpretation of theoretical and empirical studies in insect behaviour and evolution.

Introduction

Sometimes animals appear to behave in a way that does not make evolutionary sense. In the study of herbivorous insects, this is a familiar problem (Berdegue *et al.*, 1998; Cronin & Abrahamson, 2001; Mayhew, 2001); insects are attracted to particular plant species, but often the prediction that good hosts should be preferred over poor hosts and poor hosts over non-hosts is not upheld by empirical data (Stephens & Krebs, 1986; Mayhew, 1997; Ballabeni *et al.*, 2001; Scheirs & De Bruyn, 2002; West & Cunningham, 2002).

Most explanations for this mismatch between data and theory have revolved around the hypothesis that host quality (the suitability of each plant species for offspring survival) is not simply determined by a plant's nutritional quality (Jaenike, 1978; Courtney *et al.*, 1989) and additional 'missing' factors must contribute strongly to the overall fitness of the ovipositing female; these include host–plant abundance (Jaenike, 1978; Rausher, 1980; West & Cunningham, 2002), adult feeding sites (Scheirs & De Bruyn, 2002), insect learning (Cunningham & West, 2008), larval movement (Thompson, 1988; Cunningham *et al.*, 2001) and predator avoidance (Bjorkman & Larsson, 1991; Ohsaki & Sato, 1994; Ballabeni *et al.*, 2001).

An alternative explanation, however, is that nutritional quality is a good predictor of offspring survival, but the insect's host selection behaviour is limited by its ability to process sensory information – that the behaviour is somehow constrained by the mechanism. This theory has been referred to as the information processing hypothesis (IPH) (Levins & Macarthur, 1969; Bernays, 2001; Egan & Funk, 2006) with its key prediction being that generalist insects (having more information to process) should be less efficient in their responses towards host plants compared to specialists. The IPH has achieved some support from empirical studies (Janz, 2003; Egan & Funk, 2006), but to date, there has been little discussion of what these mechanisms might be or how these processing constraints might arise.

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The aim of this study is to explore how a mechanistic view of insect behaviour can be used to explore evolutionary theory. It focuses on olfaction, which plays a key role in host finding and recognition in most herbivorous insects (Bruce *et al.*, 2005; Dudareva *et al.*, 2006) and on the mechanism of the insect antennal lobe (AL), where odour information from the environment is translated into an 'odour code' and delivered to the higher centres of the insect brain.

Perhaps one of the greatest hurdles of a mechanistic theory is that it involves complex neurophysiological processes, and for many behavioural scientists this means stepping into a daunting and alien field. Olfactory processing is certainly no exception and from the outset, it is worth clarifying that the elusive odour code is likely to be an integration of many different coding mechanisms within the AL (Kuebler *et al.*, 2011). My conceptual model is a simplification of this complex process, and focuses on the most widely studied coding mechanism – spatial patterns of excitation. My aim is to explain this single coding mechanism in a way that highlights its importance in understanding insect host selection behaviour, without requiring prior knowledge of olfactory neuroscience. I begin by reviewing a number of important features of olfactory processing in the AL. I then use a simple schematic to explore how these features might influence the way host and non-host odours are perceived, and lead to constraints in the evolution of insect olfactory responses.

Understanding the olfactory mechanism: odour processing in the AL

The characteristic odour of a plant species is made up of a blend of individual compounds (volatiles). These volatiles are common to many plant species, genera and families (see reviews by Bruce *et al.*, 2005; Dudareva *et al.*, 2006; Pichersky *et al.*, 2006; Raguso, 2008).

If an herbivorous insect is to distinguish between plant odours, its olfactory system must therefore be capable of telling apart blends of chemicals that share common volatile elements. In its simplest form, an insect could distinguish between two plant odours by identifying volatiles that are present in only one of the plants (often termed 'key volatiles'). Given the enormous diversity of plant life, however, such unique, identifying volatiles are rare occurrences in nature (Bruce *et al.*, 2005), which leaves the insect's olfactory system with the more complex task of recognizing blend structure itself, i.e. the *combinations* of volatiles that co-occur in each plant odour.

Although odour identification is undoubtedly a product of the entire olfactory system, from the antennal receptors to the higher centres of the insect brain, a key hub of olfactory processing, often regarded as the 'primary centre', is the insect AL. Its function has been brought to light through pioneering research on the structure and neural wiring of the AL (Christensen *et al.*, 1991; Hansson *et al.*, 1992; Gao *et al.*, 2000; Vosshall *et al.*, 2000) and neurophysiological responses to insect pheromones and plant odours (e.g. Joerges *et al.*, 1997; Christensen *et al.*, 2000; Galizia & Menzel, 2000a,b; Carlsson *et al.*, 2002; Christensen & Hildebrand, 2002; Hansson *et al.*, 2003; Carlsson *et al.*, 2005; Menzel *et al.*, 2005; Deisig *et al.*, 2010; see Lei & Vickers, 2008 for a detailed review).

A simple schematic to conceptualize the mechanism of the AL is presented in Fig. 1. From the complex blend of many volatile compounds that make up a plants odour, a subset of volatiles (Riffell *et al.*, 2009) trigger sensory neurons [olfactory receptor neurons (ORNs)] in the insect antennae. These ORNs are volatile-specific, and each class of ORN relays information to a specific region within the AL, called a glomerulus. In this way, chemical information, relating to the volatile structure of a plant odour, is translated into spatial patterns of excitation within the AL (see Lei & Vickers, 2008). These excitation patterns are then relayed to higher centres of the insect brain via another set of neurons (projection neurons) and ultimately lead to behavioural responses, such as flying upwind towards the odour source.

For the purpose of this paper, which aims to understand how mechanism may constrain the evolution of insect host selection, two key features of the AL are summarized in the schematic: first, different plant volatiles lead to activation of different glomeruli within the AL (Joerges *et al.*, 1997; Lei & Vickers, 2008) (Fig. 1a) and second, interactions within the AL lead to *blend-specific patterns* of output firing (Joerges *et al.*, 1997; Pinero & Dorn, 2007; Deisig *et al.*, 2010; Kuebler *et al.*, 2011) (Fig 1b).

Blend-specific patterns are important because they convey contextual information – the whole (the pattern created by all volatiles together) is different from the sum of the parts (the combined patterns from each individual volatile). A recent study by Deisig *et al.* (2010) has elegantly demonstrated how spatial patterns of activity evoked by different plant odour blends are *sharper and more distinct* from one another than would occur if these patterns were simply the summed responses from individual volatiles. The key to how these blend-specific patterns are formed lies in the activity of a network of local interneurons, which connect glomeruli (Reisenman *et al.*, 2005; Olsen *et al.*, 2007; Root *et al.*, 2007; Silbering & Galizia, 2007; Olsen & Wilson, 2008; Chou *et al.*, 2010; Huang *et al.*, 2010; Seki *et al.*, 2010) (Fig. 1b), allowing activity within one glomerulus to influence activity in another. The way in which this network of interneurons achieves pattern sharpening has crucial implications towards our understanding of insect odour responses and their evolution.

Rules within the mechanism: the processing algorithm

Figure 2 presents a simple schematic for how the AL might process the odours of six plants, which differ in their suitability as hosts (for example in terms of

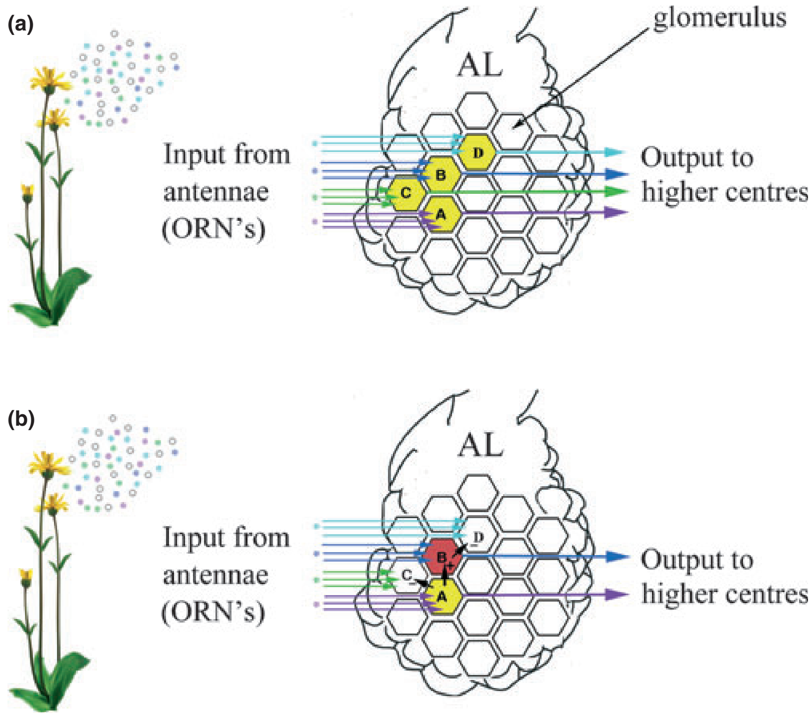

Fig. 1 How plant volatiles form spatial patterns within the antennal lobe (AL). (a) Elemental patterns. Out of the complex blend of volatile compounds that make up a plant's unique odour, a subset of volatiles (in this example, the four coloured volatiles) are detected by receptors on sensory neurons [olfactory receptor neurons (ORNs)] in the insect antennae. Different ORN classes bear different receptor types (and are thus triggered by different volatiles), and each class of ORN relays information to specific regions (called glomeruli) in the AL. For example, in this schematic, the volatile represented by the green dots activates specific (green) ORNs, which relay information to glomerulus C. As a result, blends of volatiles are translated into patterns of excitation in the AL (yellow hexagons). The activated glomeruli evoke synchronized firing in output neurons (broader arrows), which send information to higher centres of the insect brain. Further processing then leads to behavioural responses such as upwind flight towards the odour source. (b) Pattern sharpening (blend-specific patterns). When a glomerulus is activated, interneuron activity (black arrows) can influence the level of excitation in neighbouring glomeruli by increasing (+) or decreasing (−) output activity. The global response of interneurons can be represented as a processing algorithm (the example here is *A excites B, A inhibits C, B inhibits D*), which sharpens output firing patterns. Red shading denotes an increased level of glomerular activity (increased output strength) evoked by excitation from both ORNs and interneurons.

nutritional quality); Plants 1 and 2 are good hosts, Plants 3 and 4 are poor hosts and Plants 5 and 6 are non-hosts. Each plant odour is comprised of a blend of four volatiles, from a total possible pool of nine volatiles (A–I) (n.b. only volatiles that trigger ORNs, are considered here). The schematic represents the way, in nature, plants share individual volatiles, but have specific blends. As can be seen, Plants 1 and 2 have similar volatile profiles (for example they could be related species, or even interplant differences in a single species – such as with and without damage by herbivory). Poorer hosts (3 and 4) and non-hosts (5 and 6) also share the same set of volatiles (i.e. from A to I), but in different combinations.

In the schematic, each plant volatile activates a single glomerulus (hexagon) denoted by its corresponding letter. Imaging studies on the AL have shown that single volatiles often activate a number of glomeruli to different levels of excitation (Joerges *et al.*, 1997; Galizia & Menzel, 2000a; Deisig *et al.*, 2010; Carlsson *et al.*, 2011; Kuebler *et al.*, 2011), but the schematic has simplified this for the purpose of investigation. Similarly, in this schematic, each glomerulus can have only three levels of excitation: level 0 (clear) is activity below a behaviourally significant threshold, and activation level 1 (yellow) and the higher level 2 (red) lead to output to the higher centres of the insect brain. Here, level 2 can only be reached by stimulation by both incoming peripheral neurons (ORNs) and additional excitatory interneuron activity.

Figure 2a shows the spatial patterns of glomerular activity evoked by odours from the six different plants, when no interneuron effects are present. The key things to note are: first, the blend pattern is the sum of the individual volatile elements (elemental representation); second, all plant odours activate the same number of glomeruli (four) to the same level (yellow); and third, individual glomeruli cannot distinguish between plants (i.e. no one volatile, or activated glomeruli, signifies good host, poor host or non-host).

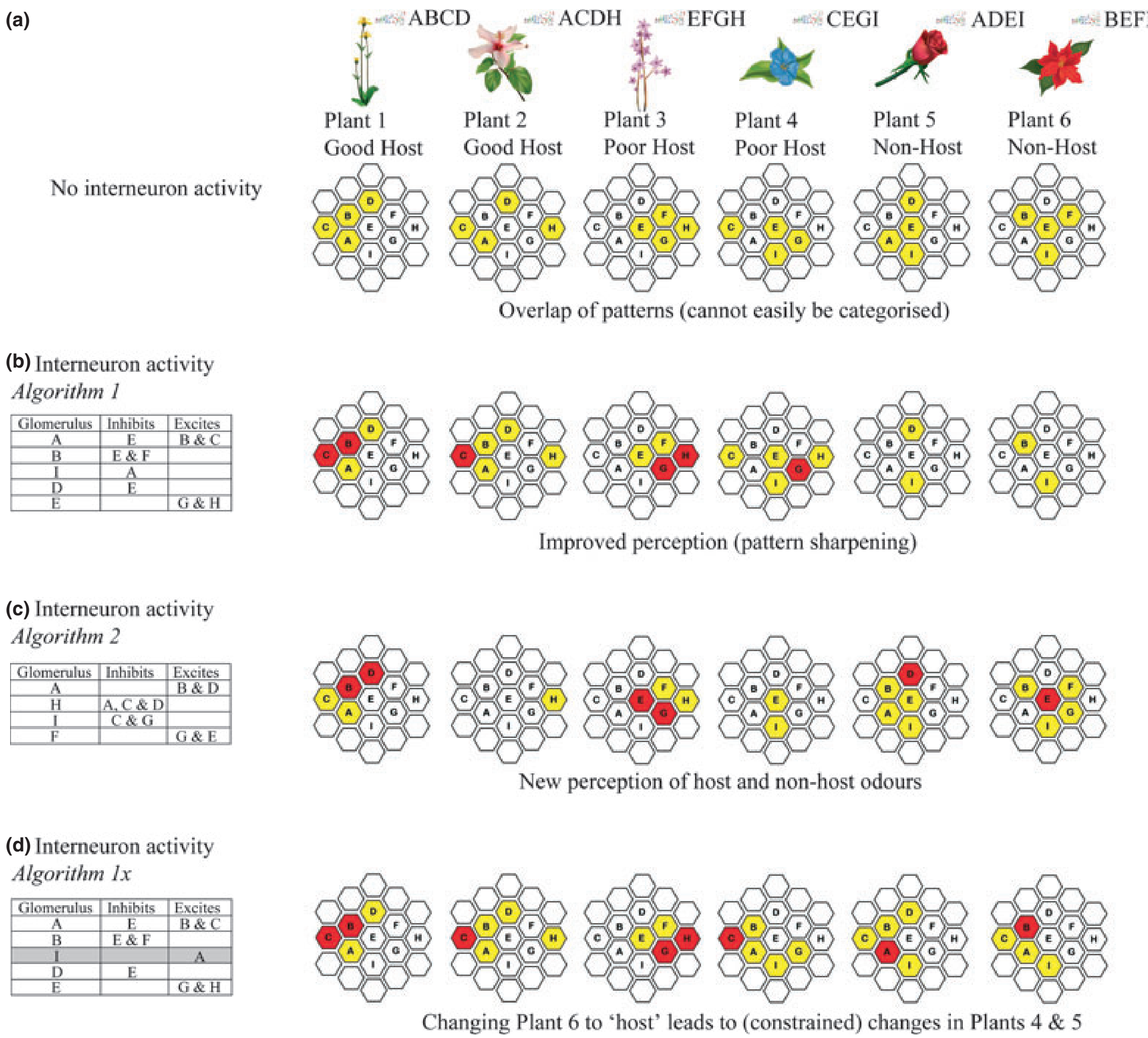

Fig. 2 How an interneuron network could influence plant odour perception in the antennal lobe (AL). The schematic represents spatial patterns in the AL evoked by odours from six plant species. Each plant odour is comprised of four volatiles from a possible 9 (A–I), and each volatile increases excitation in (activates) the corresponding lettered glomeruli. Plants 1–4 are all host species (Plants 1 and 2 are good hosts and Plants 3 and 4 are poor hosts), whereas Plants 5 and 6 are non-host species. Level of excitation: clear = activity below a threshold for recognition by higher centres of the insect brain, yellow = moderate (behaviourally relevant) excitation, red = strengthened excitation. (a) In the absence of interneuron effects (elemental representation of volatiles), host plants have overlapping patterns, which cannot be simply categorized into good, poor and non-hosts. (b) Interneuron effects detailed in Algorithm 1 give rise to sharper, more distinct patterns, which can be more easily categorized (e.g. good host = red C, poor host = red G, non-host ≤ 3 active glomeruli). (c) A new interneuron algorithm (Algorithm 2) gives rise to a different perception of host and non-host odours. (d) Simulating an adaptive change in Algorithm 1, such that Plant 6 is now perceived as a host. In Algorithm 1x, interneurons from glomerulus I now excite glomerulus A. The change in perception of Plant 6 carries the 'cost' (or constraint) of changing perception of Plant 4 (poor host to good host) and Plant 5 (non-host to host).

In Fig. 2b, interactive effects between glomeruli, simulating interneuron activity have been added. Activated glomeruli can further excite (increase by one level) and inhibit (decrease by one level) other glomeruli. For example, when glomerulus A is excited, interneuron activity increases excitation in glomeruli B and C and inhibits glomerulus E (if both excitatory and inhibitory effects occur in the same glomerulus, inhibition takes precedent – i.e. the glomerular excitation remains below a behavioural threshold).

The global AL response – the combined influence of all interneurons connecting glomeruli – can be expressed as an algorithm (i.e. if A = excited, then increase B and C and decrease E; if B = excited, then decrease E and F, etc.) This has been named Algorithm 1 (Fig. 2b). In Fig. 2c, interneuron activity has been modified by applying a new algorithm (Algorithm 2) to the schematic.

In Fig. 2d, I have considered how Algorithm 1 could be modified to include Plant 6 within the insect's range of host plants; this could be seen as an adaptive change in host selection if, for example, the insect had evolved to detoxify the defence chemicals of this plant species. The change in perception of Plant 6 is achieved by changing the influence of glomerulus I on glomerulus A (from inhibitory to excitatory). Under Algorithm 1x, volatiles from Plant 6 now evoke a 'host response', but as a consequence of this change, non-host Plant 5 is also perceived as a host and Plant 4 has moved categories from poor host to good host.

What does the schematic tell us about the benefits and constraints of the processing algorithm?

1. *Pattern sharpening*. In Fig. 2b, the patterns coded for by different plant odours are *more clearly defined* when compared with Fig. 2a: (i) there is less overlap in activated glomeruli in the good, poor and non-host categories, (ii) the strength of activation (red glomeruli) has increased in the hosts and differs in good and poor hosts and (iii) the number of activated glomeruli has been reduced (to two glomeruli) in the non-hosts.
2. *Categorization.* A simple set of rules can now identify host categories in Fig. 1b; Four or more activated glomeruli = host, less than three = non-host. Additionally, glomerular activation can define host type (activated A = good host, activated E = poor host), as can glomerular firing strength (strong red B or C = good host, strong G = poor host)
3. *Different algorithms, different patterns.* Figure 2c shows how a different algorithm can significantly change the pattern formation. Under Algorithm 2, Plants 2 and 4 now evoke weak AL responses (they are perceived as 'non-hosts') and Plants 5 and 6 evoke strong AL responses (they are perceived as 'hosts'). Thus, interneuron activity alone, without any change in glomerular number or arrangement, or incoming neuronal activity from the peripheral nervous system, can modulate the categorization of host and non-host odours. An insect with an AL that processes under Algorithm 2 has vastly different glomerular patterns evoked by the six plants and consequently perceives plant odours very differently from an insect with an AL that processes under Algorithm 1. Thus, interneurons could be a key site for evolutionary change.
4. *Volatiles common to hosts and non-host odours are still important.* From a behavioural perspective, it might appear that volatile A does not play a major role in influencing host selection – it is present in the odours of both good hosts but also in the odour of one of the non-hosts (Plant 5). From a mechanistic perspective, however, volatile A has a major influence on host recognition, through its effect on rules within the processing algorithm.
5. *Evolutionary constraints in odour perception.* In Fig. 2d, a change in perception of one non-host odour (Plant 6) is brought about by changes in interneuron activity connecting two glomeruli (I and A). Under this modification (Algorithm 1x), non-host Plant 5 is now perceived as a host and the perception of Plant 4 has changed from being perceived as a poor host (red glomerulus G) to a good host (red glomerulus C). Consequently, evolutionary change to recognize Plant 6 is constrained – it comes at the cost of changing the perception of Plants 4 and 5.
6. *Constraints and odour similarity.* The schematic also shows how the constraints that prevent Plant 6 from being perceived as a host without including Plant 5 are not merely a consequence of odour similarity. Plant 5 shares two volatiles with Plant 6 but also with Plants 1, 2 and 4. Nor are they a consequence of a particular volatile within the blend (e.g. Plants 5 and 6 both share volatile I, but so does Plant 4). Instead, constraints to evolutionary changes in perception can only be understood by considering the entire processing algorithm.

Discussion

A mechanistic view of insect olfaction could improve our understanding of why insects respond to different plant species in the way we observe. Based on current neurophysiological research, the schematic presented here is used to show how insect olfactory processing in the AL may be governed by a set of rules, or algorithm. This algorithm is inherent in the activity of spatially distinct neurophysiological units (glomeruli) and the global network of interneurons that connect them. The key implication of this view of odour processing is that changes to the perception of one odour, brought about by modification to the algorithm, will influence the processing and perception of *all* plant odours; in short, that insect responses to host and non-host odours are constrained by the olfactory mechanism.

The schematic is used to explain conceptually how glomerular activity patterns within the AL, evoked by different plant odours, can be *sharpened* by interneuron activity. This pattern sharpening allows odours to be more easily categorized into good hosts, poor hosts and non-hosts. The schematic also shows how the algorithm determines the way hosts and non-hosts are categorized; different algorithms give entirely different patterns, implying that this may be a key site for evolutionary change in odour perception.

A single-step 'evolutionary' change in the processing algorithm is used to demonstrate how modification of the response towards a single plant odour can incur the cost of re-categorizing other host and non-host species. This suggests that evolutionary biases and constraints to behaviour can exist within the processing algorithm, and that the adaptive value of a change in perception towards one plant odour would depend on concurrent changes to processing (and thus perception) of other ecologically important odours. Inter- and intraspecific differences in interneuron wiring could lead to similar observed behavioural responses towards certain host–plant species (within and between species) with differing costs of adaptation towards recognition of a new host plant. In other words, adaptation would depend more on internal mechanism than on behavioural observations of 'host preference'.

If natural selection acts upon the network of interneurons within the AL, we would predict that adaptation is towards the *best processing algorithm* – one that maximizes the total number and quality of all offspring laid on all plants and thus the best response to *all* hosts (Stephens & Krebs, 1986). The best algorithm may not, however, be one that can distinguish all host odours from non-host odours, nor one that can identify hosts relative to their individual quality (preference–performance correlations). When insects show attraction to odours of plants that do not appear to support larval development, or show stronger responses to certain 'poor' host species relative to other more suitable 'good' host species, this may occur as a constraint of the processing mechanism and does not necessarily imply that other missing factors that influence offspring fitness (such as predation and adult feeding) (Mayhew, 2001) must be sought after.

This mechanistic view of odour perception proposes that current neurophysiological evidence is in support of the IPH – neural processing may be constraining insect responses towards plant odours. The original hypothesis focuses on accuracy in host selection in generalist insects compared to specialists (Levins & Macarthur, 1969; Bernays, 2001); clearly, using this model for olfactory processing, the greater the number of plant odours that need to be distinguished among, the more constrained processing will become. A highly polyphagous insect may only be capable of broadly classifying odours, such that the majority of odours are responded to as good, poor and non-hosts. More finely tuned responses, such as preference–performance correlated responses within (e.g. decreased attraction to nutritionally deficient, or herbivore damaged plants) or between host–plant species, may only be possible in insect species with a restricted host range (Gripenberg *et al.*, 2010), where a more narrowly defined set of odours is processed.

In a recent study, Carlsson *et al.* (2011) have provided empirical evidence that perceptual differences, related to host range, may occur within the AL. These researchers measured glomerular activity patterns evoked by different plant odours, in two butterfly species – one of which was a specialist (*Aglais urticae*) and the other a generalist (*Polygonia c-album*). In general, the activity patterns in the AL were similar between the two butterfly species, but the specialist had a more specific response towards the odour of its preferred host species, compared to the generalist. In this study, however, the patterns mainly reflected input activity to the AL (i.e. before interneuron processing). A comparative study along these lines, but reflecting output activity (i.e. after processing), could generate interesting data that further support the IPH and the predictions of the theory presented here.

With the mechanism of the AL in mind, the IPH could be extended from its generalist–specialist approach to the prediction that all odour processing is constrained. Evolutionary history – host plants on which an insect species evolved (Wint, 1983; Walter & Benfield, 1994) – would play an important role in setting the algorithms coding for host odour recognition, and subsequent evolutionary change towards utilizing new hosts would depend on the fitness costs of changes to the algorithm. Adaptation involving fewer neurological changes (less genes involved) would be expected to occur more rapidly than more complex changes (Matsubayashi *et al.*, 2010). For any given insect species, processing algorithms conferring greater fitness returns may be possible (i.e. there may be algorithms that better categorize host species), but adaptation to the 'fitter' algorithm may require a substantial reorganization of neural processing (the interneuron network), with intermediate changes incurring high fitness costs. Thus, adaptive landscapes in olfactory responses, with fitness 'valleys and peaks' (Wright, 1932), would lead to host responses in polyphagous insects being relatively conservative across populations (Thompson, 1993), forming a barrier to behavioural adaptation.

The mechanistic view of olfactory behaviour has implications for the design and interpretation of empirical studies on insect–plant olfactory responses. It supports the growing body of behavioural research that has shown context, in the form of the co-occurrence of volatiles in odour blends, is crucial to understanding insect olfactory responses (Pinero & Dorn, 2007; Riffell *et al.*, 2009; Beyaert *et al.*, 2010; Tasin *et al.*, 2010; Webster *et al.*, 2010). From a mechanistic perspective, the 'role' of a volatile in host recognition is inherent in the way it influences the global response, or processing algorithm. Volatiles should not be seen as attractants or deterrents in themselves. Empirical studies may show that certain volatiles are common to a host or non-host species, but this does not imply that these volatiles have an independent effect on behaviour. Similarly, studies that demonstrate the release of particular volatiles by herbivore-damaged plants should be cautious with implications that volatiles have inherent ecological roles.

The validity of the theory presented here depends heavily on the role of the AL in sharpening, categorizing and coding odour information before presenting it to higher centres of the insect central nervous system (CNS). Natural selection may act on any part of the olfactory mechanism, and adaptations in peripheral responses and central responses would also be expected under selection pressures in the environment (Ramdya &

Benton, 2010), affecting input to the AL and interpretation of AL activity. Down-streaming effects from the higher centres of the insect brain (e.g. through learning) may also influence processing within the AL (Faber *et al.*, 1999; Denker *et al.*, 2010).

Although much of the structure and functioning of the AL has been uncovered in recent years, precisely how odour blends are translated into an odour code is still an area of intense discussion (Lei & Vickers, 2008; Silbering *et al.*, 2008; Martin & Hildebrand, 2010; Seki *et al.*, 2010). Here, the emphasis is on the most widely examined mechanism in the AL, spatial patterns of excitation, but it should be borne in mind that other coding mechanisms, such as synchrony, frequency and latency of neuron firing, may also carry inherent information on odour composition and quality (Lei & Vickers, 2008; Martin & Hildebrand, 2010; Kuebler *et al.*, 2011). Computer models and simulations capable of integrating these multiple dimensions of coding could be the next important step in understanding odour processing and odour perception in the AL. These simulations could be used to generate predictions, such as expected behavioural responses to different volatile combinations that could then be tested empirically. By bringing together theoretical and empirical studies on mechanism with observed behavioural responses, we may move towards a more complete understanding of insect–plant interactions and their evolution.

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References

Ballabeni, P., Wlodarczyk, M. & Rahier, M. 2001. Does enemy-free space for eggs contribute to a leaf beetle's oviposition preference for a nutritionally inferior host plant? *Funct. Ecol.* **15**: 318–324.

Berdegue, M., Reitz, S.R. & Trumble, J.T. 1998. Host plant selection and development in Spodoptera exigua: do mother and offspring know best? *Entomol. Exp. Appl.* **89**: 57–64.

Bernays, E.A. 2001. Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annu. Rev. Entomol.* **46**: 703–727.

Beyaert, I., Waeschke, N., Scholz, A., Varama, M., Reinecke, A. & Hilker, M. 2010. Relevance of resource-indicating key volatiles and habitat odour for insect orientation. *Anim. Behav.* **79**: 1077–1086.

Bjorkman, C. & Larsson, S. 1991. Pine sawfly defense and variation in host plant resin acids – a trade-off with growth. *Ecol. Entomol.* **16**: 283–289.

Bruce, T.J., Wadhams, L.J. & Woodcock, C.M. 2005. Insect host location: a volatile situation. *Trends Plant Sci.* **10**: 269–274.

Carlsson, M.A., Galizia, C.G. & Hansson, B.S. 2002. Spatial representation of odours in the antennal lobe of the moth *Spodoptera littoralis* (Lepidoptera : Noctuidae). *Chem. Senses* **27**: 231–244.

Carlsson, M.A., Knusel, P., Verschure, P. & Hansson, B.S. 2005. Spatio-temporal Ca2+ dynamics of moth olfactory projection neurones. *Eur. J. Neurosci.* **22**: 647–657.

Carlsson, M.A., Bisch-Knaden, S., Schapers, A., Mozuraitis, R., Hansson, B.S. & Janz, N. 2011. Odour maps in the brain of butterflies with divergent host–plant preferences. *PLoS ONE* **6**: e24025.

Chou, Y.H., Spletter, M.L., Yaksi, E., Leong, J.C.S., Wilson, R.I. & Luo, L.Q. 2010. Diversity and wiring variability of olfactory local interneurons in the Drosophila antennal lobe. *Nat. Neurosci.* **13**: 439–449.

Christensen, T.A. & Hildebrand, J.G. 2002. Pheromonal and host-odor processing in the insect antennal lobe: how different? *Curr. Opin. Neurobiol.* **12**: 393–399.

Christensen, T.A., Mustaparta, H. & Hildebrand, J.G. 1991. Chemical communication in heliothine moths. 2. Central processing of intraspecific and interspecific olfactory messages in the male corn-earworm moth helicoverpa-zea. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **169**: 259–274.

Christensen, T.A., Pawlowski, V.M., Lei, H. & Hildebrand, J.G. 2000. Multi-unit recordings reveal context-dependent modulation of synchrony in odor-specific neural ensembles. *Nat. Neurosci.* **3**: 927–931.

Courtney, S.P., Chen, G.K. & Gardner, A. 1989. A general-model for individual host selection. *Oikos* **55**: 55–65.

Cronin, J.T. & Abrahamson, W.G. 2001. Goldenrod stem galler preference and performance: effects of multiple herbivores and plant genotypes. *Oecologia* **127**: 87–96.

Cunningham, J.P. & West, S.A. 2008. How host plant variability influences the advantages to learning: a theoretical model for oviposition behaviour in Lepidoptera. *J. Theor. Biol.* **251**: 404–410.

Cunningham, J.P., West, S.A. & Zalucki, M.P. 2001. Host selection in phytophagous insects: a new explanation for learning in adults. *Oikos* **95**: 537–543.

Deisig, N., Giurfa, M. & Sandoz, J.C. 2010. Antennal lobe processing increases separability of odor mixture representations in the honeybee. *J. Neurophysiol.* **103**: 2185–2194.

Denker, M., Finke, R., Schaupp, F., Grun, S. & Menzel, R. 2010. Neural correlates of odor learning in the honeybee antennal lobe. *Eur. J. Neurosci.* **31**: 119–133.

Dudareva, N., Negre, F., Nagegowda, D.A. & Orlova, I. 2006. Plant volatiles: recent advances and future perspectives. *Crit. Rev. Plant Sci.* **25**: 417–440.

Egan, S.P. & Funk, D.J. 2006. Individual advantages to ecological specialization: insights on cognitive constraints from three conspecific taxa. *Proc. R. Soc. B Biol. Sci.* **273**: 843–848.

Faber, T., Joerges, J. & Menzel, R. 1999. Associative learning modifies neural representations of odours in the insect brain. *Nat. Neurosci.* **2**: 74–78.

Galizia, C.G. & Menzel, R. 2000a. Odour perception in honeybees: coding information in glomerular patterns. *Curr. Opin. Neurobiol.* **10**: 504–510.

Galizia, C.G. & Menzel, R. 2000b. Probing the olfactory code. *Nat. Neurosci.* **3**: 853–854.

Gao, Q., Yuan, B.B. & Chess, A. 2000. Convergent projections of Drosophila olfactory neurons to specific glomeruli in the antennal lobe. *Nat. Neurosci.* **3**: 780–785.

Gripenberg, S., Mayhew, P.J., Parnell, M. & Roslin, T. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. *Ecol. Lett.* **13**: 383–393.

Hansson, B.S., Ljungberg, H., Hallberg, E. & Lofstedt, C. 1992. Functional specialization of olfactory glomeruli in a moth. *Science* **256**: 1313–1315.

Hansson, B.S., Carlsson, M.A. & Kalinova, B. 2003. Olfactory activation patterns in the antennal lobe of the sphinx moth, *Manduca sexta*. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **189**: 301–308.

Huang, J., Zhang, W., Qiao, W.H., Hu, A.Q. & Wang, Z.R. 2010. Functional connectivity and selective odor responses of excitatory local interneurons in Drosophila antennal lobe. *Neuron* **67**: 1021–1033.

Jaenike, J. 1978. On optimal oviposition behaviour in phytophagous insects. *Theor. Popul. Biol.* **14**: 330–356.

Janz, N. 2003. The cost of polyphagy: oviposition decision time vs error rate in a butterfly. *Oikos* **100**: 493–496.

Joerges, J., Kuttner, A., Galizia, C.G. & Menzel, R. 1997. Representations of odours and odour mixtures visualized in the honeybee brain. *Nature* **387**: 285–288.

Kuebler, L.S., Olsson, S.B., Weniger, R. & Hansson, B.S. 2011. Neuronal processing of complex mixtures establishes a unique odor representation in the moth antennal lobe. *Front. Neural Circuits* **5**: 7.

Lei, H. & Vickers, N. 2008. Central processing of natural odor mixtures in insects. *J. Chem. Ecol.* **34**: 915–927.

Levins, R. & Macarthur, R. 1969. An hypothesis to explain the incidence of monophagy. *Ecology* **50**: 910–911.

Martin, J.P. & Hildebrand, J.G. 2010. Innate recognition of pheromone and food odors in moths: a common mechanism in the antennal lobe? *Front. Behav. Neurosci.* **4**: 159.

Matsubayashi, K.W., Ohshima, I. & Nosil, P. 2010. Ecological speciation in phytophagous insects. *Entomol. Exp. Appl.* **134**: 1–27.

Mayhew, P.J. 1997. Adaptive patterns of host–plant selection by phytophagous insects. *Oikos* **79**: 417–428.

Mayhew, P.J. 2001. Herbivore host choice and optimal bad motherhood. *Trends Ecol. Evol.* **16**: 165–167.

Menzel, R., Galizia, C.G., Muller, D. & Szyszka, P. 2005. Odour coding in projection neurons of the honeybee brain. *Chem. Senses* **30**(Suppl. 1): i301–i302.

Ohsaki, N. & Sato, Y. 1994. Food plant choice of pieris butterflies as a trade-off between parasitoid avoidance and quality of plants. *Ecology* **75**: 59–68.

Olsen, S.R. & Wilson, R.I. 2008. Lateral presynaptic inhibition mediates gain control in an olfactory circuit. *Nature* **452**: 956–960.

Olsen, S.R., Bhandawat, V. & Wilson, R.I. 2007. Excitatory interactions between olfactory processing channels in the Drosophila antennal lobe. *Neuron* **54**: 89–103.

Pichersky, E., Noel, J.P. & Dudareva, N. 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. *Science* **311**: 808–811.

Pinero, J.C. & Dorn, S. 2007. Synergism between aromatic compounds and green leaf volatiles derived from the host plant underlies female attraction in the oriental fruit moth. *Entomol. Exp. Appl.* **125**: 185–194.

Raguso, R.A. 2008. Wake up and smell the roses: the ecology and evolution of floral scent. *Ann. Rev. Ecol. Evol. Syst.* **39**: 549–569.

Ramdya, P. & Benton, R. 2010. Evolving olfactory systems on the fly. *Trends Genet.* **26**: 307–316.

Rausher, M.D. 1980. Host abundance, juvenile survival and oviposition preference in *Battus philenor*. *Evolution* **34**: 342–355.

Reisenman, C.E., Christensen, T.A. & Hildebrand, J.G. 2005. Chemosensory selectivity of output neurons innervating an identified, sexually isomorphic olfactory glomerulus. *J. Neurosci.* **25**: 8017–8026.

Riffell, J.A., Lei, H., Christensen, T.A. & Hildebrand, J.G. 2009. Characterization and coding of behaviorally significant odor mixtures. *Curr. Biol.* **19**: 335–340.

Root, C.M., Semmelhack, J.L., Wong, A.M., Flores, J. & Wang, J.W. 2007. Propagation of olfactory information in Drosophila. *Proc. Natl Acad. Sci. USA* **104**: 11826–11831.

Scheirs, J. & De Bruyn, L. 2002. Integrating optimal foraging and optimal oviposition theory in plant-insect research. *Oikos* **96**: 187–191.

Seki, Y., Rybak, J., Wicher, D., Sachse, S. & Hansson, B.S. 2010. Physiological and morphological characterization of local interneurons in the Drosophila antennal lobe. *J. Neurophysiol.* **104**: 1007–1019.

Silbering, A.F. & Galizia, C.G. 2007. Processing of odor mixtures in the Drosophila antennal lobe reveals both global inhibition and glomerulus-specific interactions. *J. Neurosci.* **27**: 11966–11977.

Silbering, A.F., Okada, R., Ito, K. & Galizia, C.G. 2008. Olfactory information processing in the Drosophila antennal lobe: anything goes? *J. Neurosci.* **28**: 13075–13087.

Stephens, D.W. & Krebs, J.R. 1986. *Foraging Theory*. Princeton University Press, Princeton.

Tasin, M., Backman, A.C., Anfora, G., Carlin, S., Ioriatti, C. & Witzgall, P. 2010. Attraction of female grapevine moth to common and specific olfactory cues from 2 host plants. *Chem. Senses* **35**: 57–64.

Thompson, J.N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol. Exp. Appl.* **47**: 3–14.

Thompson, J.N. 1993. Preference hierarchies and the origin of geographic specialization in host use in swallowtail butterflies. *Evolution* **47**: 1585–1594.

Vosshall, L.B., Wong, A.M. & Axel, R. 2000. An olfactory sensory map in the fly brain. *Cell* **102**: 147–159.

Walter, G.H. & Benfield, M.D. 1994. Temporal host–plant use in 3 polyphagous heliothinae, with special reference to helicoverpa-punctigera (Wallengren) (Noctuidae, Lepidoptera). *Aust. J. Ecol.* **19**: 458–465.

Webster, B., Bruce, T., Pickett, J. & Hardie, J. 2010. Volatiles functioning as host cues in a blend become non-host cues when presented alone to the black bean aphid. *Anim. Behav.* **79**: 451–457.

West, S.A. & Cunningham, J.P. 2002. A general model for host plant selection in phytophagous insects. *J. Theor. Biol.* **214**: 499–513.

Wint, W. 1983. The role of alternative host–plant species in the life of a polyphagous moth, operophtera-brumata (Lepidoptera, Geometridae). *J. Anim. Ecol.* **52**: 439–450.

Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding and selection in evolution. *Proc. Sixth Internat Congr. Genetics Ithaca New York* **1**: 356–366.

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"Ours is a world of sights and sounds. We live by our eyes and ears and tend generally to be oblivious to the chemical happenings in our surrounds. Such happenings are ubiquitous. All organisms engender chemical signals, and all, in their respective ways, respond to the chemical emissions of others. The result is a vast communicative interplay, fundamental to the fabric of life.."

THOMAS EISNER
AND JERROLD MEINWALD
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