NCCR PLANT SURVIVAL

Plant Survival: A Story of Sex, Violence and Light

Final meeting of the NCCR *Plant Survival*
23-24 January 2013, Neuchâtel, Switzerland

speakers

- *John Pickett*
- *Robert Raguso*
- *Spencer Barrett*
- *Antoine Guisan*
- *Sam Zeeman*
- *Céline Besagni*
- *Matthias Erb*
- *Mark van Kleunen*
- *Jeroen Stuurman*
- *Christian Fankhauser*

ABSTRACT BOOK

FINAL MEETING

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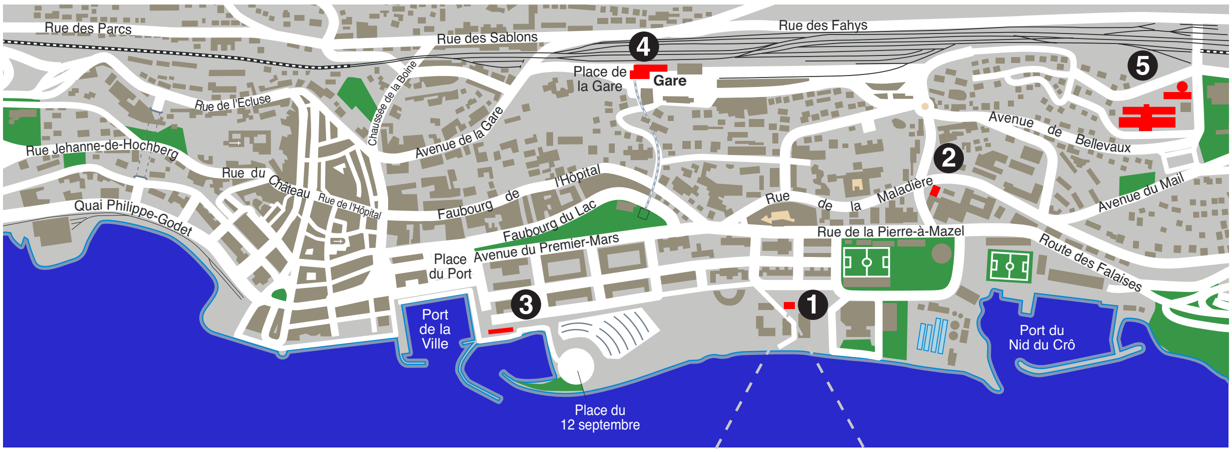

❶ Aula des Jeunes-Rives

❷ Restaurant *Le Romarin*

❸ Hotel *Beaulac*

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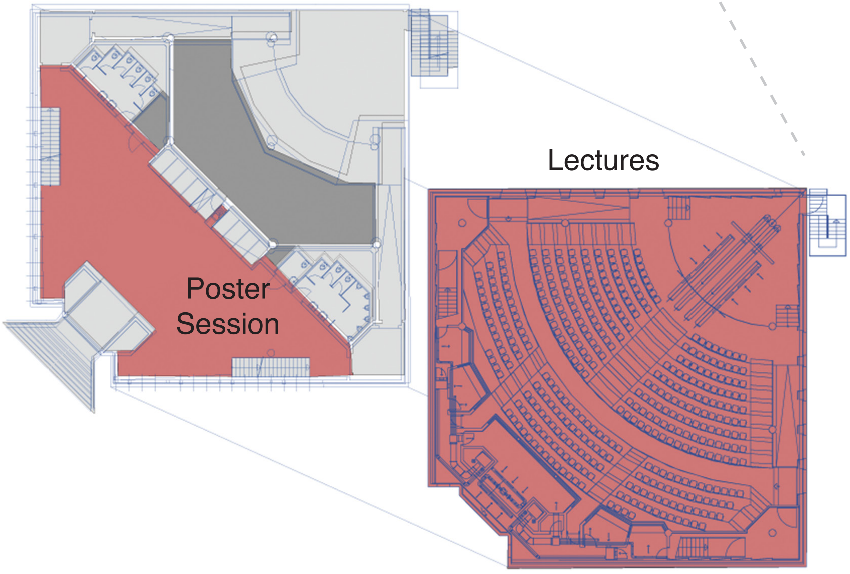


Programme

Plant Survival: a story of sex, violence and light

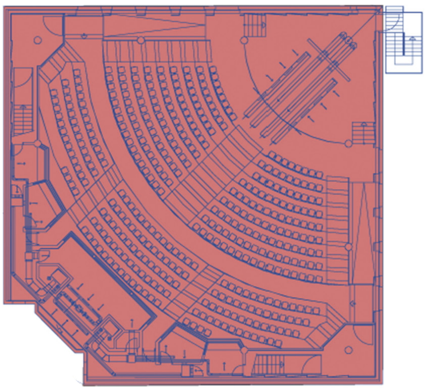
Wednesday 23 January 2013:
Topic *SEX* (in the morning), Topic *LIGHT* (in the afternoon)

08:00 – 09:30	Registration desk
09:30 – 09:50	Opening ***Martine Rahier / Ted Turlings***
09:50 – 10:30	***Spencer Barrett*** Evolution of sex ratios and gender strategies in flowering plants
10:30 – 11:00	Coffee break
11:00 – 11:40	***Robert Raguso*** Measuring selection on scent: the next frontier for plant volatile research
11:40 – 12:20	***Jeroen Stuurman*** Plant breeding with green gene mutations
12:20 – 14:00	Lunch at the restaurant *Le Romarin*
14:00 – 14:40	***Céline Besagni*** Role of plastoglobules in the synthesis and accumulation of vitamin E and K
14:40 – 15:20	***Christian Fankhauser*** Sensing light direction: signal transduction during phototropism in *Arabidopsis*
15:20 – 17:00	coffee break and poster session
17:00 – 17:40	***Sam Zeeman*** A journey down a metabolic pathway, leading to discoveries in plant development
19:00	Social dinner (*Hôtel Beaulac*)

Thursday 24 January 2013: Topic *VIOLENCE* (in the morning)

09:00 – 09:40	***John Pickett*** Plant survival using new GM and elicitor based technologies
09:40 – 10:20	***Matthias Erb*** Chemical signals as determinants of root-herbivore interactions
10:20 – 11:00	coffee break
11:00 – 11:40	***Antoine Guisan*** How far can we understand and anticipate plant invasions? Lessons learned from *Centaurea stoebe*
11:40 – 12:20	***Mark van Kleunen*** The success formula of invasive green aliens
12:30	Announcement of poster prizes and Lunch at the restaurant *Le Romarin*

LECTURES



Evolution of sex ratios and gender strategies in flowering plants

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Despite a strong theoretical basis, there is currently little known about the mechanisms governing sex-ratio variation in plants. Here, I present new results from our studies of sex ratios and gender strategies in flowering plants. I begin by presenting the results of a large-scale comparative analysis of the ecological and genetic correlates of sex-ratio variation in dioecious angiosperms. Using comparative analyses of 243 species, representing 123 genera and 61 families, it was found that male-biased sex ratios were twice as common as female-biased ratios and were associated with long-lived growth forms, biotic seed dispersal and fleshy fruits. Female bias was associated with abiotic pollen dispersal and sex chromosomes. I then discuss studies on the mechanisms governing female-biased sex ratios in *Rumex*, a genus of wind-pollinated herbs. I show that females located in close proximity to males produced more strongly female biased sex ratios compared to more isolated females. These results suggest that male proximity may influence progeny sex ratios by affecting pollen loads and the strength of gametophytic competition. Finally, I present recent studies of gender strategies and sex-ratio variation in the clonal aquatic macrophyte *Sagittaria latifolia*. Using genetic markers I demonstrate a close correspondence between sex ratios based of ramets and genets and that clone sizes in monoecious populations are significantly larger than in dioecious populations. I also document near continuous variation in sex phenotype frequencies ranging from monoecy through subdioecy to dioecy and demonstrate that subdioecy in this species can arise via two distinct genetic mechanisms. The striking sexual diversity in *S. latifolia* demonstrates that sex expression can be largely a quantitative trait and warns against forcing species into typological categories of sexual system, as commonly practised by most taxonomists.

Measuring selection on scent: the next frontier for plant volatile research

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A growing number of studies address the natural variation and functional significance of floral volatiles; recent work has established the importance of floral scent in reproductive isolation, pollinator shifts, enhancement of outcrossing and floral defense against natural enemies. Nevertheless, few studies directly measure Lande-Arnold selection gradients on floral scent, either to determine its effects on seed fitness (female function) or siring success (male function). Here I summarize the lessons learned from three such studies in which the relationship of seed fitness to floral scent was contrasted directly with traditional floral traits (colour and/or shape).

In the first study[1], colour polymorphic flowers of *Hesperis matronalis* were augmented with floral scent extracts collected during day or night. Stronger, nocturnal extracts increased seed fitness when augmented diurnally, due to increased pollination by syrphid flies, regardless of floral colour. In the second study[2], both ants (nectar thieves) and bumblebees (pollinators) were repelled by high nectar concentrations of 2-phenylethanol in skypilot flowers (insert). Scented nectar increased pollen limitation on seed fitness and thus indirectly intensified bee-mediated selection on floral shape. In the third study[3], an unbiased approach was taken to calculate selection on floral traits of *Penstemon digitalis*, resulting in positive selection on floral scent but not on floral size or colour. The target of selection was linalool, the only nectar-soluble volatile in these flowers. Taken together, these studies highlight the potential for floral volatiles to mediate several aspects of plant reproductive fitness, from pollinator attraction to defense.

Skypilot *Polemonium viscosum* flower being pollinated by a *Bombus balteatus* bee in the Rocky Mountains (USA)

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1 Galen C, et al. 2011. Dosage-dependent impacts of a floral volatile compound on pollinators, larcenists and the potential for floral evolution in the Alpine Skypilot *Polemonium viscosum*. ***American Naturalist*** 177: 258-272.

2 Parachnowitsch AL, Raguso RA, Kessler A 2012. Phenotypic selection to increase floral scent emission, but not flower size or colour in bee-pollinated *Penstemon digitalis*. ***New Phytologist*** 195: 667-675.

3 Majetic CJ, Raguso RA, Ashman T-L 2009. The sweet smell of success: floral scent affects pollinator attraction and seed fitness in *Hesperis matronalis*. ***Functional Ecology*** 23: 480-487.

Plant breeding with green gene mutations

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Molecular research on plant genes and their functions is fueled by an implicit promise: that we may adapt them for the improvement of our crop plants. However, despite the many known genes/functions, their direct use in practical plant breeding is seen relatively seldom.

To resolve this disparity, the challenge lies in the identification and/or creation of gene-variants (alleles) that outperform the resident alleles in the elite germplasm. In a breeder's context, plant biotechnology is an optimization problem at the level of single genes.

Keygene aims to provide effective non-GMO solutions to this problem. We build upon multi-faceted technology platforms : (1) high-throughput structure-function analysis of natural and designed alleles, (2) random mutagenesis to create allelic series, (3) systematic and standardized phenotyping, (4) targeted nucleotide exchange for precision modification in planta.

Using these technologies, and the associated proprietary knowledge, we work together with breeding companies to unlock the potential of molecular genetics for growing better crops.

Role of plastoglobules in the synthesis and accumulation of vitamin E and K

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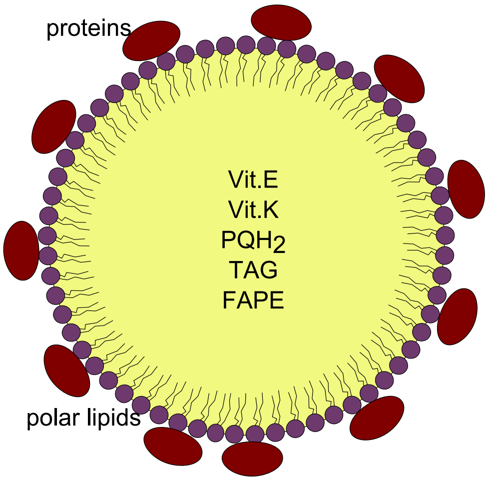
Plastoglobules (PG) are lipoprotein particles ("lipid droplets") in photosynthetic organisms. They constitute a sub-organellar compartment in plastids such as chloroplasts, chromoplasts and leucoplasts.

Structurally related to the cytoplasmic lipid droplet family that store triacylglycerols in their core and originate from the endoplasmic reticulum, PG are delimited by a polar lipid monolayer continuous with the thylakoid outer membrane from which they emerge by a "blistering" mechanism. Secondary blistering events at the surface of existing PG result in the formation of interconnected grape like PG clusters observed in stressed chloroplasts. The physical connections between thylakoid membrane and PG suggests that bidirectional lipid trafficking occurs at the places of contact.



PG structural model

PG are easily isolated by flotation on a sucrose gradient, because they contain only small amounts of protein while the interior is filled with neutral lipids. Among these are a variety of antioxidant molecules belonging to the prenylquinone family: plastoquinol (PQH_2) and tocopherol (Vitamin E) are major constituents whereas phylloquinone (Vitamin K) is also present but in minor concentrations. Triacylglycerols (TAG) and fatty acids phytyl esters (FAPE) are also present in significant amounts.

During conditions provoking oxidative stress (e.g high light, nitrate starvation, drought) or during senescence, leading to an alteration of the thylakoid membrane, the size and the number of PG increase due to the accumulation of lipids in their hydrophobic core. For many years, the observation that PG accumulate antioxidant molecules or catabolic products contributed to the idea that they represented a passive lipid storage site for the plastid under stress. However, the analysis of the PG proteome has changed this idea. In addition to structural proteins called fibrillins (or plastoglobulins), enzymes involved in various lipid metabolic pathways and uncharacterized proteins are present. The proteins are localized at the surface of the PG and are in contact with the head group of the polar lipids. These findings strongly suggest an active implication of PG in plant lipid metabolism.

Sensing light direction: signal transduction during phototropism in *Arabidopsis*

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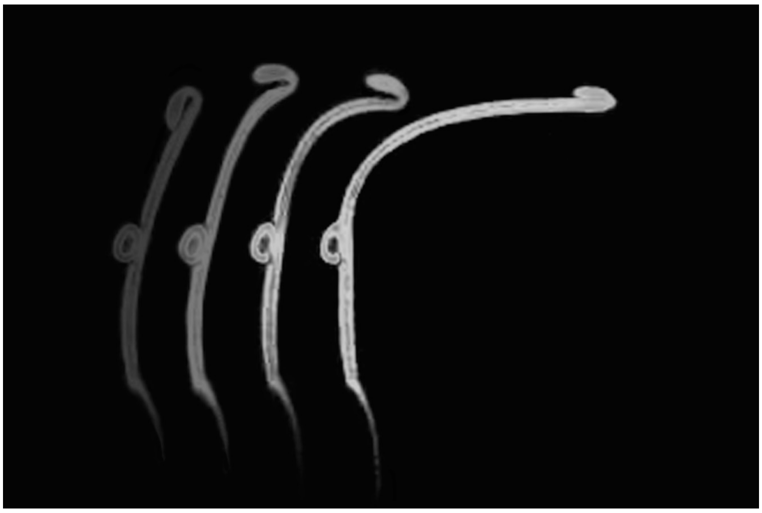
Phototropin blue-light receptors (phot1 and phot2) in *Arabidopsis* activate a range of light regulated responses, including phototropism, leaf movements, stomatal opening, leaf expansion, and chloroplast movements. Those responses generally serve to optimize photosynthesis and allow the plant to adapt to changing light environments. Phototropins are light-regulated protein kinases that are broadly expressed and present at the plasma membrane in the dark. Blue light induces their protein kinase activity and leads to internalization of a fraction of the photoreceptor. In order to understand the steps leading from photoreceptor activation to asymmetric hypocotyl growth leading to phototropism we are addresing the following questions : (1) Where does phot1 perceive the light signal that activates phototropism? Does the photoreceptor act cell autonomously, or does the response involve transportation of a signal from the site of light perception to the site of action? (2) Is light-induced phot1 translocation from the plasma membrane to the cytosol a mechanism of desensitization or is its transport into the cytosol essential for signaling? (3) What are the substrates of phot1 kinase activity and how does phosphorylation regulate these targets. I will discuss our latest findings on these questions.



A journey down a metabolic pathway, leading to discoveries in plant development

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The research of my group has for many years focused on starch - a key plant product for our society and a major product of photosynthesis in chloroplasts. Starch is comprised of glucans that form semi-crystalline granules. In leaves, it is made during the day from photo-assimilated carbon and degraded again at night to support metabolism. During the course of the NCCR we have contributed several new discoveries that advanced the understanding of starch metabolism, particularly g enzymes. However, the phosphate groups can subsequently obstruct enzymes of starch degradation, hence the need for their concomitant removal. The key enzymes of starch degradation are beta-amylases, which generate maltose for export to the cytosol of the cell via a unique chloroplast envelope transporter, MEX1. Our research of the pathway of starch degradation has thrown up a few surprises and prompted new research programmes. Firstly, our studies have revealed several genes in plant encoding proteins that are homologous to enzymes of starch degradation, but which are non-catalytic. The most striking example is that of beta-amylase-like proteins residing in the cell nucleus and controlling gene expression. The loss or over-expression of these proteins influences plant morphology, not metabolism. We propose that they are sugar sensors helping to integrate metabolic signals to control the pattern of growth and development. We are actively researching this topic now. Secondly, glucan-binding phosphatases (probably resulting from convergent evolution) exist in mammals and are involved in glycogen dephosphorylation. The loss of this phosphatase - Laforin - is known to cause a fatal neurological disorder (Lafora disease) in which starch-like particles accumulate in brain cells. However, the biological significance of glycogen phosphorylation is not really understood, even in a simple bacterial system like *E. coli*. We have engaged in collaboration with scientists working in microbial and animal systems to advance this area.

Plant survival using new GM and elicitor based technologies

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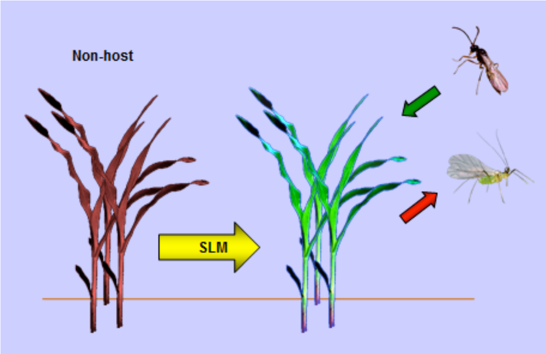
Pioneering scientific research on stress induced plant defence has underpinned development of natural products as patented elicitors of plant resistance to insect pests. Such elicitors, including cis-jasmone[1], are not only showing value in the field, for example against wheat pests, but studies on their mode of action[2,3] are also providing elicitor-responsive promoters that can be used to activate heterologously expressed defence genes, even for sentinel plants. Genes associated with the biosynthesis of secondary plant metabolites giving indirect defence, and those related to insect defence pheromones, are now targeted for delivery by genetically modified crop plants. Already, the first phase of field trials with wheat, genetically modified to produce the aphid alarm pheromone, have been conducted in the UK based on the original demonstration of this technology in the model plant *Arabidopsis thaliana*[4].

For the future, attempts will be made to combine this with elicitor-responsive promoters so as to mimic, more closely, aphid release of the pheromones. While other elicitors and new GM technologies are being advanced, work from the push-pull programme in Africa, in which lepidopterous stemborer moths are repelled from cereal crops by intercrops which also attract natural enemies, with a trap crop used to attract oviposition, is demonstrating the value of these semiochemical based approaches. In addition, elicitors have been discovered[5] that are produced by stemborer moth eggs, particularly those of *Chilo partellus*, which cause systemic release of semiochemicals attracting not only egg parasitoids, but also larval parasitoids in a "smart" anticipation of that development. These genetics are present in landraces and farmer varieties, gathered seasonally as seed for resowing[6], but are not present in elite hybrid varieties. Some of the hypotheses underpinning these studies have provided opportunities for developing new approaches for repelling carnivorous arthropods from human and other animal hosts[7].



Small lipophilic molecule (SLM) acting as an elicitor of plant defence.

1 Bruce TJA, Matthes MC, Chamberlain K, et al. 2008. cis-Jasmone induces Arabidopsis genes that affect the chemical ecology of multitrophic interactions with aphids and their parasitoids. ***PNAS*** 105: 4553-4558.
2 Matthes MC, Bruce TJA, Ton J, et al. 2010. The transcriptome of cis-jasmone-induced resistance in Arabidopsis thaliana and its role in indirect defence. ***Planta*** 232: 163-1180.
3 Matthes M, Bruce T, Chamberlain K, et al. 2011. Emerging roles in plant defense for cis-jasmone-induced cytochrome P450 CYP81D11. ***Plant Signaling & Behavior*** 6: 1-3.
4 Beale MH, Birkett MA, Bruce TJA, et al. 2006. Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behaviour. ***PNAS*** 103: 10509-10513.
5 Tamiru A, Bruce T, Woodcock C, et al. 2011. Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. ***Ecology Letters*** 14: 1075-1083.
6 Tamiru A, Bruce TJA, Midega CAO, et al. 2012. Oviposition induced volatile emissions from African smallholder farmers' maize varieties. ***Journal of Chemical Ecology*** 38: 231-234.
7 Pickett JA, Birkett MA, Logan JG 2008. DEET repels ORNery mosquitoes. ***PNAS*** 105: 13195-13196.

Chemical signals as determinants of root-herbivore interactions

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Root herbivores are among the most harmful agricultural pests. Yet, how they interact with their host plants is a vastly understudied area of chemical ecology. With the support of the NCCR "Plant Survival", we have investigated the behavioural and chemical interplay between maize (*Zea mays*) and the western corn rootworm (*Diabrotica virgifera virgifera*), a highly specialized root herbivore, and our research highlights why *D. virgifera* is such a successful and damaging pest. First we found that *D. virgifera* does not only use CO_2 as a host location cue below ground, but that it can also integrate root-herbivore-induced (*E*)-β-caryophyllene and leaf-herbivore-suppressed ethylene as additional signals to select suitable host plants[1,2]. Second, once *D. virgifera* has located a host plant, it can tolerate and exploit benzoxazinoids, toxic secondary metabolites that are exuded by maize roots, to orient itself within the root system and feed on the most nutritious tissues[3]. Third, although *D. virgifera* attack does induce a transcriptional defense response in maize roots, the plants do not become more resistant, but more susceptible against subsequent attack. Fourth, *D. virgifera* avoids the negative effects of leaf-herbivore induced root resistance by arriving early in the season and specifically detecting and moving away from leaf-infested plants. Evidently, *D. virgifera* is very well equipped to deal with constitutive and induced maize defenses, and we propose that breeding for tolerance rather than resistance may help to reduce its negative impact on agricultural productivity.

Can it be beaten? *Diabrotica virgifera* is extremely well adapted to its host plant. Picture: Matthias Held & Ivan Hiltpold.

1 Robert CAM, Erb M, Duployer M, Zwahlen C, Doyen GR, Turlings TCJ 2012. Herbivore-induced plant volatiles mediate host selection by a root herbivore. ***New Phytologist*** 194(4): 1061-1069.
2 Robert CAM, Veyrat N, Glauser G, Marti G, Doyen GR, Villard N, Gaillard MDP, Köllner TG, Giron D, Body M, Babst BA, Ferrieri RA, Turlings TCJ, Erb M 2012. A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. ***Ecology Letters*** 15: 55-64.
3 Robert CAM, Erb M, Hibbard B, French W, Zwahlen C, Turlings TCJ 2012. A specialist root herbivore reduces plant resistance and uses an induced plant volatile to aggregate in a density dependent manner. ***Functional Ecology***. DOI: 10.1111/j.1365-2435.2012.02030.x

How far can we understand and anticipate plant invasions? Lessons learned from *Centaurea stoebe*

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and Petitpierre B [1]

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By analyzing a large data set on occurrences of successful plant invaders in their native and introduced range, we showed that macroclimatic niche shifts are rare in terrestrial plant invaders. Our model species, *Centaurea stoebe*, is a remarkable exception. It experienced a significant niche expansion during its invasion from Europe into North America, notably towards drier and warmer habitats, thus representing a great opportunity to elucidate potential underlying ecological and/or evolutionary mechanisms. Our joint efforts and multi-pronged approaches allowed dissecting the relative contribution of both pre-adaption and rapid evolutionary changes along two distinct invasion routes in North America, making our plant invasion model system one of the best studied ever. Knowledge gained from our findings have now been used by our consortium to successfully develop new large-scale research programs on plant invasions and their management and are currently used to design an online support tool for Swiss nature managers.

The success formula of invasive green aliens

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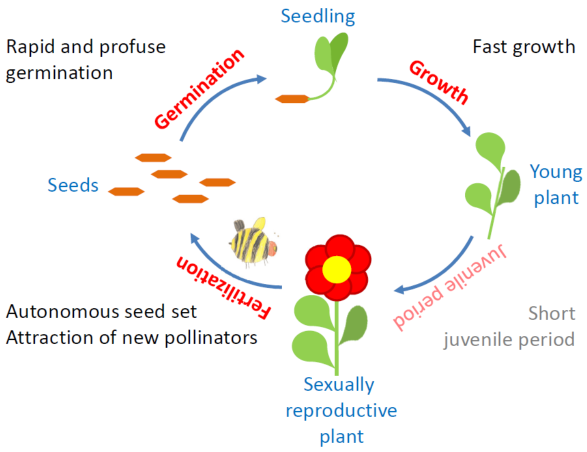
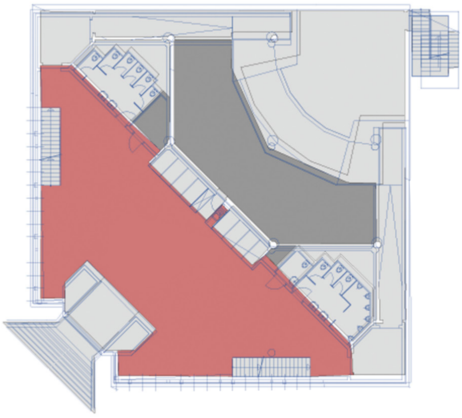
Humans have intentionally, and sometimes accidentally, introduced thousands of plant species from their native regions to other parts of the world. Some of those introduced alien species have established naturalized populations, and some of those have become invasive. A major question in ecology is what determines the establishment success of alien plant species. We use a multi-species comparative approach to answer this question. Although there is no universal success formula, the results of our studies indicate that several characteristics are frequently associated with naturalization and invasion success of alien species[1]. These characteristics include among others rapid and profuse seed germination[2,3], fast growth[4], the ability to take advantage of increased nutrient levels[5,6], and the ability of autonomous seed set[7,8] or the ability to attract many pollinators. In conclusion, fast, independent and flexible species that take advantage of their opportunities are among the successful alien species.



Some characteristics that appear to be part of the success formula of invasive plant species

1 van Kleunen M, Weber E, Fischer M 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. ***Ecology Letters*** 13: 235-245.
2 van Kleunen M, Johnson SD 2007. South African Iridaceae with rapid and profuse seedling emergence are more likely to become naturalized in other regions. ***Journal of Ecology*** 95: 674-681.
3 Schlaepfer DR, Glättli M, Fischer M, van Kleunen M 2010. A multi-species experiment in their native range indicates pre-adaptation of invasive alien plant species. ***New Phytologist*** 185: 1087-1099.
4 Dawson W, Fischer M, van Kleunen M 2010. Maximum relative growth rate of common UK plant species is positively associated with their global invasiveness. ***Global Ecology and Biogeography*** 20: 299-306.
5 Dawson W, Fischer M, van Kleunen M 2012. Common and rare plant species respond differently to fertilisation and competition, whether they are alien or native. ***Ecology Letters*** 15: 873-880.
6 Dawson W, Rohr RP, van Kleunen M, Fischer M 2012. Alien plant species with a wider global distribution are better able to capitalize on increased resource availability. ***New Phytologist*** 194: 859-867.
7 van Kleunen M, Manning JC, Pasqualetto V, Johnson, SD 2008. Phylogenetically independent associations between autonomous self-fertilization and plant invasiveness. ***American Naturalist*** 171: 195-201
8 Hao JH, Qiang S, Chrobock T, van Kleunen M, Liu QQ 2011 A test of Baker's Law: breeding systems of invasive species of Asteraceae in China. ***Biological Invasions*** 13: 571-580.

POSTERS



Induced resistance and the memory effect: further understandings and its applicability in crop protection

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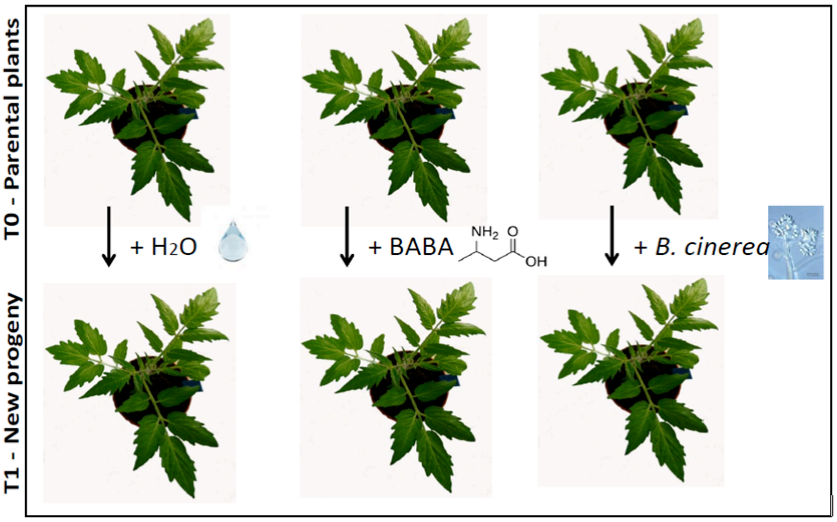
Plants throughout evolution have developed very sophisticated defense mechanisms against biotic and abiotic stress. These stresses influence plants in different ways, such as affecting their physiology or altering their resistance responses. Plant resistance can be enhanced by the use of different natural and synthetic components that will induce “priming”. A primed plant will respond faster and stronger when confronted with a biotic or abiotic stress. Interestingly, primed plants do not show major trade-offs in growth and seed production. Furthermore, recent studies in our laboratory have shown that descendants of plants that had been exposed to stress showed an increased priming and resistance response when confronted with pathogens.

The first part of my project is to further understand how the non-protein amino acid β-aminobutyric acid (BABA) acts as a chemical elicitor enhancing plant defense against pathogens in *Arabidopsis thaliana* and further understand the transgenerational induced resistance also in the model plant.

The second part of my project will be dedicated to validate the results already obtained with the model plant *Arabidopsis thaliana* in a crop plant such as tomato variety Micro-tom, where I will also investigate the behavior of descendants of primed plants against biotic stresses. This project will contribute to our understanding of this phenomenon towards its applicability in agriculture as a crop protection strategy.



Scheme of transgenerational lines produced in Micro-tom

Seasonal switch between intra-specific competition and facilitation in invasive *Alliaria petiolata* supports stress gradient hypothesis

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Space and time are the two fundamental drivers of ecological dynamics. Yet, the Stress Gradient Hypothesis (SGH) —which predicts that the patterns of intra- and inter-specific interactions shift from negative to positive with increasing environmental stress—conceptualises stress predominantly from a spatial perspective. We asked: do the predictions of the SGH at the intraspecific level hold in relation to temporal, e.g. seasonal, environmental stress? We conducted a density removal experiment, which was complemented by a two-year field survey, involving the non-native biennial forb *Alliaria petiolata* at the Koffler Scientific Reserve—Ontario, Canada. We found—in both experimental and field studies—statistically significant negative density-dependent survival in summer and positive density-dependent survival over winter. Patterns were stronger in the experiment than in natural populations, where *A petiolata* occurred with other species. Our results suggest that the SGH at the intra-specific level is applicable to seasonal variation in environmental stress, though our ability to detect its effect in natural communities may depend on other factors such as species dominance and environmental heterogeneity.

Contrasting climatic niche dynamics during the Eastern and Western invasions of spotted knapweed in North America

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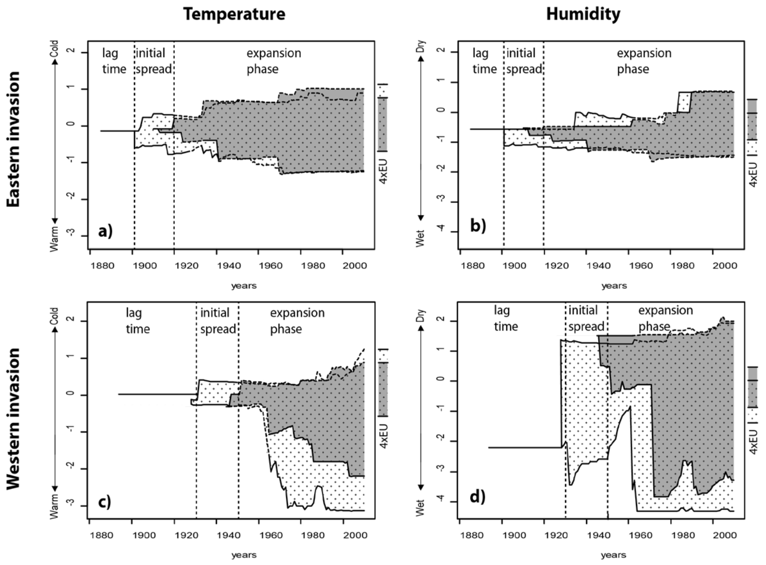
Spotted knapweed (*Centaurea stoebe*) is a well-studied plant native to Europe and highly invasive in North America. The species was introduced in the late 1890s almost simultaneously to the eastern and western coast of North America, but the subsequent inland invasions from these two entry ports showed very different patterns of spread both in terms of velocity and habitats colonized. Here we model the spatio-temporal dynamic of the realized climatic niche limits of the two invasions using historical and climatic data. During the eastern invasion, the realized niche gradually increased over time until reaching niche limits similar to the native range. Conversely, in the West the niche abruptly expanded after an extended time lag, colonizing climatic conditions not occupied in the native range, although present. We discuss these differences in the temporal dynamics of the niche patterns in the light of historical, ecological and evolutionary processes evidenced for this species. We suggest that both pre-adaptation and rapid evolutionary change contributed to the invasion success of *C. stoebe* in North America. In the western invasion, a longer time lag was needed to conquer the novel habitat due to climatic isolation. Invasion into the dry rangeland was then favored by enemy release and the less competitive vegetation, combined with post-introduction evolutionary change underlying the increased population growth rate as compared to European *C. stoebe*.



Dynamic of niche limits through time - Left and right panels show niche change along a temperature and humidity gradient. (a-b) niche limits through time for Eastern populations, (c-d) for Western. Lines show the 2.5% upper and lower quantiles. Solid lines indicate significant differences in niche limits between populations in natural (in gray) and ruderal habitats (dotted areas). Tick marks on the right side of the plots indicate the native niche limits.

Genetics of chemical defence traits in European *Populus*

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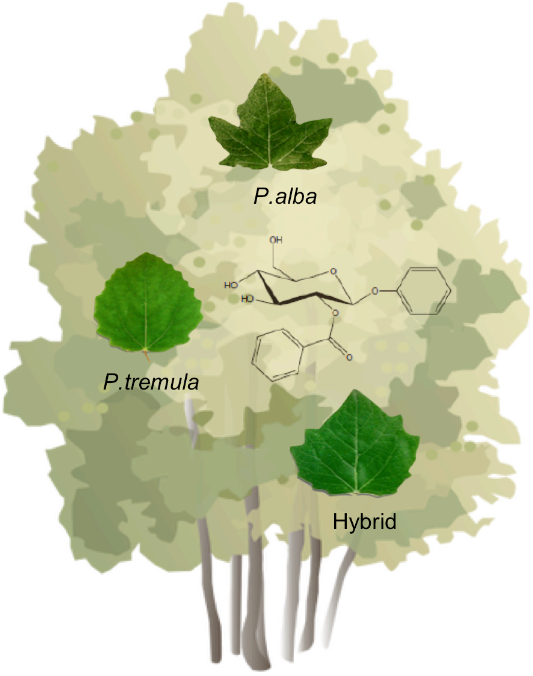
Longlived plants such as trees cannot move to escape environmental challenges such as herbivores, pathogens or climate change. Defence mechanisms can have a direct impact on their fitness and survival. Hybrid zones often exhibit a greater abundance and diversity of herbivores, but interspecific recombination also opens the opportunity to combine multiple defence mechanisms from each parental species.

We studied patterns of expression of thirty-four key secondary metabolites involved in biotic and abiotic defence (Salicinoids, Flavonoids, and Chlorogenic acids) in three large natural hybrid zone of two ecologically divergent European *Populus* species, *Populus alba* and *Populus tremula*. Uni- and multivariate analysis of metabolomic profiles in recombinant hybrids reveals the potential and limits of wide recombination to generate novel combinations of functionally important traits[1]. Admixture mapping in natural hybrids reveals the complex genetic architecture of the studied traits. We will discuss the implications of our findings for the genetics of species differences, chemical ecology, and potential community and ecosystem effects of the studied traits and their underlying genes.



Populus as a study system for chemical ecology, speciation genetics, and community genetics.

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[1] Caseys C, Glauser G, Stölting K, Christe C, Albrectsen B, Lexer C. 2012. Effects of interspecific recombination on functional traits in trees revealed by metabolomics and genotyping-by-resequencing. ***Plant Ecology & Diversity***: In press, Doi: 10.1080/17550874.2012.748850

Long distance defense signaling in *Arabidopsis thaliana*: a key role for LOX6

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Mechanical wounding activates the production of jasmonate both in and near the wound and in distal connected leaves[1,2]. The jasmonate pathway is initiated in plastids by 13-lipoxygenases (13-LOXs), enzymes that oxygenate tri-unsaturated fatty acid to provide the first precursors for jasmonate which controls defense against both invertebrates and vertebrates[3,5]. Four 13-LOXs have the potential to initiate the jasmonate synthesis[6]. A recent study described a role for LOX2 in leaf defense against herbivory[2] while LOX3 and LOX4 are known to be essential for male fertility[7]. The biological role for LOX6 is unknown. In this present study we identified a role of LOX6 in the defense of young leaves near the apical meristem in response to herbivory. Interestingly, in bioassay comparing the WT and *lox6A*, *Spodoptera littoralis* weight gain was not affected. Indeed, when all 13-LOXs except LOX6 were down-regulated (in the *lox2-1 lox3B lox4A* mutant) larvae preferred older leaves to plant centre, whereas they fed in an inverse manner on the quadruple mutant (*lox2-1 lox 3B lox 4A lox6A*). Concomitant with these observations the expression pattern of 13-LOX6 was stronger in plant centre and mainly localized in a specific cell type in the vasculature. All evidence suggests that, through long distance signalling, herbivores activate JA synthesis through LOX6 in the region of the apical meristem.

Caterpillar feeding on an *Arabidopsis* leaf.

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2 Glauser G, Dubugnon L, Mousavi SAR, Rudaz S, Wolfender JL, et al. 2009. Velocity Estimates for Signal Propagation Leading to Systemic Jasmonic Acid Accumulation in Wounded Arabidopsis. ***Journal of Biological Chemistry*** 284: 34506-34513.

3 Acosta IF, Farmer EE 2010. Jasmonates. ***The Arabidopsis Book***: 1-13. doi:10.1199/tab.0129.

4 Mafli A, Goudet J, Farmer EE 2012. Plants and tortoises: mutations in the *Arabidopsis* jasmonate pathway increase feeding in a vertebrate herbivore. ***Molecular Ecology*** 21: 2534-2541.

5 Kessler A, Halitschke R, Baldwin IT 2004. Silencing the Jasmonate Cascade: Induced Plant Defenses and Insect Populations. ***Science*** 305: 665-668.

6 Bannenberg G, Martinez M, Hamberg M, Castresana C 2009. Diversity of the Enzymatic Activity in the Lipoxygenase Gene Family of Arabidopsis thaliana. ***Lipids*** 44: 85-95.

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Arabidopsis thaliana cytochrome P450 CYP89A9 is involved in the formation of novel major chlorophyll catabolites during leaf senescence

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Nonfluorescent chlorophyll catabolites (NCCs) have been described as end products of chlorophyll breakdown in *Arabidopsis*[1]. NCCs are open tetrapyrroles formed from respective fluorescent precursors, termed FCCs, through acid catalyzed isomerization after import of FCCs into the vacuoles of senescing cells. In *Arabidopsis*, quantification of NCCs in senescent leaves had implied that their amount might correspond to the pool of chlorophyll present in green leaves[2].

Here we present genetic and biochemical evidence for CYP89A9, a cytochrome P450, to be involved in the formation of another type of nonfluorescent chlorophyll catabolite. *cyp89a9* mutants accumulate 10 times more NCCs than wild-type plants. Comparison between profiles of colorless catabolites in wild-type and *cyp89a9* mutants allowed the identification of unknown catabolites in wild-type that are absent in *cyp89a9* mutants. Chemical characterisation of these unknown catabolites reveals that they are dioxobilane-type NCCs (DNCCs). DNCCs are similar to NCCs except that the formyl group attached to pyrrole B in NCCs is absent. Like most other cytochromes P450s, CYP89A9 seems to localize to the endoplasmic reticulum with its catalytic site facing the cytosol suggesting that FCCs are the in vivo substrate of CYP89A9. We therefore tested in vitro activity of CYP89A9 on FCCs and showed that recombinant CYP89A9 expressed in insect cells is able to convert FCCs to a novel fluorescing product which is indicated to be decarbonylated. Thus, we propose that CYP89A9 is involved in FCCs decarbonylation in *Arabidopsis*. The fluorescing products (DFCCs) are then imported into the acidic vacuole and isomerized to DNCCs. Moreover, due to 10 fold increase of NCCs in *cyp89a9* mutants, we propose, in contrast to our earlier finding[2], that DNCCs represent the majority of the total chlorophyll catabolites accumulating in *Arabidopsis*.

1 Hörtensteiner S, Krautler B 2010. Chlorophyll breakdown in higher plants. ***Biochim Biophys Acta***
2 Pružinská A, Tanner G, Aubry S, Anders I, Moser S, Müller T, Ongania K-H, Kräutler B, Youn J-Y, Liljegren SJ, Hörtensteiner S 2005. Chlorophyll breakdown in senescent Arabidopsis leaves: characterization of chlorophyll catabolites and of chlorophyll catabolic enzymes involved in the degreening reaction. ***Plant Physiol.*** 139: 52-63.

Testing the relative roles of competition and plant-soil feedbacks in explaining commonness and rarity of native and alien plant species

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Alien invasive and native common plant species may reach a high abundance because the difference between intraspecific and interspecific competition effects is smaller compared to non-invasive and rare species. In other words, invasive and common species (hereafter ‘common’) may be less self-limiting at higher intraspecific relative frequency than non-invasive and rare species (hereafter ‘rare’). In addition, abundance of plant species has been linked to the strength of plant-soil feedbacks, with common species sometimes exhibiting less negative plant-soil feedbacks compared to rare native plant species. This indicates that commonness may also be at least partially explained by resistance to soil pathogens and relaxed plant-soil feedbacks, allowing common species to reach higher intraspecific densities than rare species. However, no study to date has tested for differences in plant-soil feedbacks between invasive and non-invasive alien species and between common and rare native species simultaneously, in an explicitly density-dependent context with intra- and interspecific competition.

It might be expected that plant-soil feedbacks and intraspecific competition at high density both regulate the abundance of native and alien plant species in a similar manner. However, alien species as a whole may suffer from less negative plant soil feedback effects than native species, if they have escaped soil enemies present in their native range. Initial experiments, however, demonstrate little difference between Central European alien and native plant species in the strength of plant-soil feedback affecting them. A comparative, multi-species approach involving both alien and native plant species is outlined, which will ascertain whether plant-soil feedbacks and/or intraspecific competition explain variation in abundance of both alien and native plant species.

Do teosinte plants benefit from recruiting parasitoid wasps?

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Parasitoid wasps use volatiles that are emitted by plants in response to herbivore attack as a means to find their hosts. It has therefore been proposed that the volatile emissions are part of an indirect defense strategy to recruit parasitoids and other natural enemies of the attackers. This proposed signalling function of herbivore-induced plant volatiles is still controversial because there is little evidence that plants benefit from the attraction of natural enemies, particularly in the case of parasitoids as they do not immediately kill the herbivores. The aim of this study was to evaluate the importance of attracting parasitoid wasps for plant growth and survival in teosinte, the wild ancestor of maize.

In a natural setting in Mexico, the country of origin of maize, we planted teosinte in large field cages, in the presence or absence of second-instar larvae of the moth *Spodoptera frugiperda*, one of the most devastating pests of maize in the Americas, and female parasitoids of the species *Campoletis sonorensis*, an important co-occurring natural enemy. We assessed larval parasitism, plant volatile emission, plant damage and plant survival.


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Parasitoid *Campoletis sonorensis* oviposits in herbivore *Spodoptera frugiperda*.

The results show that the presence of only small numbers of parasitoid wasps dramatically reduced the damage inflicted by the larvae and that, for young plants, this resulted in a reduction in plant mortality. These results support the notion that plants benefit from recruiting parasitoid wasps and may help to settle the discussion on the defensive function of herbivore-induced volatiles, which has received considerable attention lately.

3D models of study organisms to produce personalized images for presentations: is there a demand among reseachers?

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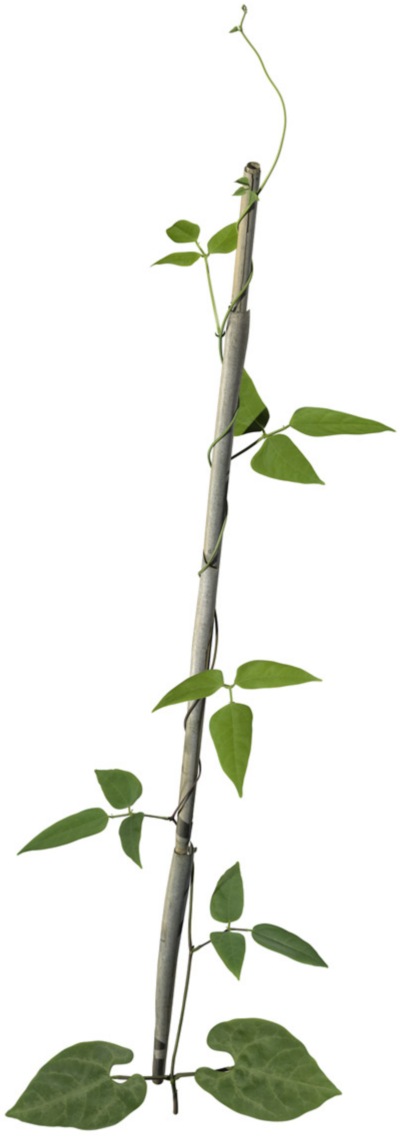
When it comes to communicating the results of a successful research project, visual elements play an important role to pass on the message. Photographs do often a good job for introducing the study organism, but sometimes clutter-free illustrations without distracting background may be preferable to tell the story. When they are accomplished, traditional illustrations are rather difficult to adapt to new purposes. By contrast, images rendered with 3D technology are much more flexible in use. Once a 3D model of an organism has been created, many of its properties remain editable and can even be animated, e.g. shape (morphing), color as well as other surface characteristics such as specularity and transparency. Copies of a 3D model (clones) must not look exactly the same as they can by rotated and viewed from a different perspective.

Yet, the downside of the 3D technology is the fact that sculpting and texturing a model, which contains additional depth information, is still a more time-consuming process than drawing or painting a picture, which makes 3D illustrations unaffordable for the average scientist, whose budget is meant for research and not for luxury artwork, after all. Potentially this problem could be solved, if researchers studying the same organism join to cover the production costs of a 3D model, while still benefitting from individualized images - the rendering of variants is a task that can be left to the computer. However, some basic questions are still open: Is there sufficient demand for such images in the first place? Are people willing to pay enough for it to make it a viable business? And if yes, how are these market opportunities best exploited?



Lima bean *Phaseolus lunatus*

Apoplastic pH regulation during phototropism

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Plants have the ability to perceive light direction and adapt their growth accordingly, which optimizes photosynthetic yield. Although this process called phototropism has been observed for decades, the molecular mechanisms underlying it are still not completely elucidated. It is known that phototropins are the major photoreceptors that control this response. Downstream of the phototropin signalling takes place a lateral redistribution of the plant growth hormone auxin, leading to asymmetric growth. Nevertheless, it remains elusive which exact mechanisms are responsible for gradient formation.

We recently discovered a potential novel mechanism. Based on computational modelling approach we propose that differential cell wall pH regulation is a crucial factor for establishing auxin gradient. Combinations of genetic, physiological and pharmacological studies have been conducted to support this model.

Impact of non-host herbivores on a plant-parasitoid interaction: a test of the robustness of infochemicals

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Plant volatiles produced in response to herbivory are of key importance for the foraging behavior of parasitoids in search of hosts. Despite the large amount of studies documenting such plant-parasitoid interactions, very few studies have investigated the robustness of plant infochemical signals to disturbances by feeding non-host herbivores. Here, using as a model the plant *Brassica rapa*, the herbivore *Pieris brassicae* and its parasitoid *Cotesia glomerata*, all three native species, we compared the attractiveness of plants damaged by *P. brassicae* to *C. glomerata* females, with plants damaged by 1) non-host herbivores, and 2) non-host herbivores and *P. brassicae* simultaneously. Overall, we tested nine species of non-host herbivores, both native (*Mamestra brassicae*, *Plutella xylostella*, *Athalia rosae*, *Phylotreta nemorum*, *Phylotreta cruciferae*) and exotic (*Spodoptera littoralis*, *Spodoptera exigua*, *Heliothis virescens*, *Myzus persicae*) in a 4-arm olfactometer setting.

Results demonstrated that 1) *C. glomerata* females show a strong preference for plants damaged by their host compared to plants damaged solely by a non-host herbivore, independently of the origin of the non-host; plants damaged by a non-host are, however, overall more attractive than undamaged plants. In addition, 2) effects of simultaneous herbivory are origin dependent: plants damaged by both the host and an exotic non-host herbivore are considerably less attractive than plants damaged by the host alone. On the other hand, plants damaged by the host and a native non-host are overall as attractive as plants damaged by the host alone. These results suggest that the infochemicals emitted by *B. rapa* in response to *P. brassicae* are specific and that the parasitoid *C. glomerata* has evolved a high ability to distinguish them from an those emitted in response to a variety of other herbivores. *C. glomerata* is also robust to infochemical disturbances associated with simultaneous multiple herbivory, but only for native non-host herbivores. This suggests that *C. glomerata* could not adapt to disturbances caused by herbivores it did not coexist with, and that invasive exotic herbivores have the potential to impact chemically mediated plant-parasitoid interactions in nature.

The native parasitoid *Cotesia glomerata*

Effect of maize domestication on the growth of *Spodoptera* sp.

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It is widely accepted that domestication and agronomic selection on plants for agricultural purpose may be responsible for the loss of defenses. To test this assumption on maize, the performance, in terms of growth, of three different pest species of the genus *Spodoptera* (Lepidopteran: Noctuidae) has been compared on cultivated maize and its wild ancestor, teosinte. The maize-specialist feeder (*S. frugiperda*) had similar performance on both plants. But the generalist feeder species (*S. exigua* and *S. littoralis*) had significantly reduced performance when feeding on teosinte compared to maize. The use of maize lines introgressed with teosinte genome has shown that some specific parts of this genome, that have been lost during domestication process, may be responsible for the observed difference of performance. This results fits with the initial statement that the domestication process have altered maize-herbivore interactions, enhancing the performance of some pest species.

Manipulating fragrance production in *Petunia* to understand a pollination syndrome

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To be pollinated, plants have multiple floral traits such as color, odor, nectar and morphology of the flower to attract pollinators. Differences in these floral traits can lead to specific interactions with pollinators causing reproductive isolation of a phenotype and ultimately speciation. The tobacco hornworm, *Manduca sexta*, feeds on *Petunia axillaris* which produces white and benzenoid-scented flowers. On the other hand, *P. exserta* with a hummingbird pollination syndrome produces scentless red flowers. These two species of *Petunia* were crossed to isolate quantitative trait loci (QTLs) responsible for odor production in *Petunia* and to measure the importance of this trait in floral sensory ecology of *Manduca*. Two QTLs that define the difference in production of benzenoid volatiles between the two species were identified by interspecific crossings. The sensitivity of *Manduca*'s antennae to the fragrance of *Petunia* was measured using gas chromatography linked electroantennography to identify active flower volatiles. Reciprocal introgressions of scent QTLs were used in choice experiments in a wind tunnel to estimate the influence of odor production on the choices made by foraging moths. The *Manduca* antenna is very sensitive to certain volatile compounds particularly benzenoids. Choice experiments demonstrate that *M. sexta* prefers scented plants and that scent determines choice at a short distance. When exposed to conflicting cues of colour versus scent, *Manduca* displays no preference, indicating that colour and scent provide equivalent cues. Our results highlight the importance of scent for the plant-pollinator interactions in the context of the pollination syndrome of *Petunia*. The genetic basis underlying such a major phenotypic difference in flower types appears to be relatively simple and may enable rapid loss or gain of scent through hybridization in the wild.

A *Manduca sexta* feeding on the scented *Petunia axillaris*.

Increased population growth in invasive polyploid *Centaurea stoebe* in a common garden

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Biological invasions are inherently demographic processes, but trait differences between native and introduced genotypes are rarely linked to population growth rates. The native European plant *Centaurea stoebe* occurs as two cytotypes with different life histories: monocarpic diploids (EU 2x) and polycarpic tetraploids (EU 4x). However, in its introduced range in North America so far only tetraploids (NA 4x) have been found. Using a novel experimental common garden approach with artificial populations, we compared the demographic performance of the three geo-cytotypes (EU 2x, EU 4x, NA 4x) in the presence and absence of the specialist root-mining insect herbivore *Agapeta zoegana*. With data collected over a period of three years, we parameterized periodic matrix models and conducted elasticity analyses and life table response experiments (LTREs). We found no difference in population growth rate between the two European cytotypes and no significant effects of herbivory in all geo-cytotypes. However, there was a pronounced increase in population growth rate for North American compared to European tetraploids due to increased seed production and juvenile establishment. These results suggest that genetic drift or rapid evolution, rather than pre-adaptation through polyploidy may explain the invasion success of tetraploids.

See also: Hahn MA, Buckley YM, Müller-Schärer H 2012. Increased population growth rate in invasive polyploid *Centaurea stoebe* in a common garden. ***Ecology Letters*** 15: 947-954.

Tight genetic linkage of prezygotic barrier loci creates a multifunctional speciation island in *Petunia*

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Flower color, scent and reproductive organ morphology are major features of pollination syndromes and are under strong selective pressure due to floral visitors. So far, genetic studies have focused on single attraction traits such as color and behavioral studies demonstrated how single loci condition shifts in pollinator attraction. However, the modification of a pollination syndrome requires coordinated changes in all relevant traits and changes in allele frequencies due to selection on one trait generate a correlated selection response in other traits. This implies that either genes of individually large or pleiotropic effect, or linked clusters of genes with large cumulative effect, may play a role in the evolution of reproductive isolation and speciation.

Petunia is a classical genetic model system and comprises species that attract different pollinators, making it ideally suited to study the genetics underlying pollinator driven speciation. We identified tight genetic linkage of loci specifying five major pollination syndrome traits: floral scent production, UV absorption, visible colour, pistil- and stamen length between the red, hummingbird-pollinated *P. exserta* and the white nocturnal hawkmoth pollinated *P. axillaris*. Comparison of the genetic arrangement with other Solanaceae indicated that *P. exserta* and *P. axillaris* specifically clustered loci important for these floral traits into a multifunctional speciation island. Furthermore, this functional speciation island is located in an area with low recombination frequency that supports linkage disequilibrium between the isolating loci. This has important evolutionary implications. (1) Linkage of several isolating loci has a ‘multiplier effect’ on reproductive isolation; (2) linkage disequilibrium prevents it from dissolution; (3) the co-segregation of floral traits allows for rapid switches between pollination syndromes when confronted with shifts in pollinator availability.

The rare species *Petunia exserta* with exserted sexual organs, adapted to hummingbird pollination. From left to right: *P. exserta*; *P. axillaris* pollinated by *Hyles lineata*, Uruguay 2008; *P. exserta* (lab line) pollinated by *Hylocharis chrysura* during choice experiments, Uruguay 2009.

Encapsulation of entomopathogenic nematodes

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Soil-dwelling insects can cause problematic damages to crops. The western corn rootworm (WCR) (*Diabrotica virgifera virgifera*) is responsible for damage estimated to 2 billion US$. In order to combat these pests, we plan to use polymer-based capsules as a novel and ecologically sound way to introduce biological control agents into the soil. Polymer-based capsules can hold liquids and thus allow the encapsulation of entomopathogenic nematodes (EPN). In order to target the pests, the capsule may contain attractants and feeding stimulants to lure the pests by deviating them away from the plant to the capsule. Additionally, we will include in the capsules an active constituent from green pea roots that put nematodes in a state of quiescence in order to cope with conservation and infection timing issues.

Responses of maize to insect herbivores: compensatory growth following leaf attack?

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Plants react to herbivore-attack by increasing the production of jasmonate-dependent defenses, including secondary metabolites and inhibitory proteins. The jasmonate burst and the cost of induced defenses leads to a reduction in growth in many plants, either via hormonal-cross talk or resource trade-offs. However, a number of studies indicate that plants may also compensate for tissue loss by increasing their growth upon herbivore attack. Until today, the mechanisms and specificity of herbivore-induced growth compensation remain poorly described. With this in mind, we investigated how the growth of young maize plants (*Zea mays* var. B73) is affected by two leaf feeders, the generalist caterpillar *Spodoptera littoralis* and the well-adapted maize pest *Spodoptera frugiperda*. Plants fed upon for 48 h by *S. littoralis* had a lower shoot dry weight than uninfested control plants, while plants attacked by *S. frugiperda* tended to have a higher shoot mass. To test whether this enhanced growth is induced by physical damage to the leaves or insect-derived compounds, we collected oral secretions (OS) from both caterpillar species, which were applied onto wounded leaves of different plants. After 4 days of treatment, plants treated with *S. frugiperda* OS had a higher shoot dry weight than untreated controls and wounded plants receiving water instead of OS. Shoot mass of *S. littoralis* OS-treated plants did not differ from the controls. These findings suggest that compound(s) present in oral secretions of the specialist *S. frugiperda*, but not of the generalist *S. littoralis*, induce compensatory plant growth. Root biomass was similar in all treatment groups; therefore, increased biomass above ground is unlikely to occur at the expense of reduced root growth. As *S. frugiperda* attack also leads to the induction of typical defensive processes, maize plants seem to be able to overcome any possible growth-defense trade-offs and increase both defenses and growth upon attack by an adapted herbivore. As *S. frugiperda* is resistant to many maize defenses, induced compensatory growth may be an alternative strategy of the plant to reduce the negative effects of *S. frugiperda* attack in an agricultural setting.

Matthias Held

Spodoptera littoralis (top) and *S. frugiperda* (bottom) feeding on maize leaves

Compatibility of transgenic legumes and natural enemies to control bruchids (Coleoptera: Chrysomelidae)

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Bruchid beetles (Coleoptera: Chrysomelidae) are important pests of stored legume seeds and difficult to manage. Genetic engineering has been used to transfer an α-amylase inhibitor (αAI-1) from the common bean into other legumes to protect their seeds against important bruchid pests.

The presence of αAI-1 in transgenic chickpea (*Cicer arietinum*) and cowpea (*Vigna unguiculata*) seeds significantly increases their resistance to sensitive bruchids such as *Callosobruchus* spp.[1] In contrast, the bruchid species *Acanthoscelides obtectus* and *Zabrotes subfasciatus* are not affected by αAI-1. A powerful approach to control also such tolerant species might be the combination of αAI-1 transgenic seeds with hymenopteran parasitoids. However, parasitoid larvae or host-feeding females might ingest the inhibitor when attacking resistant bruchid larvae that contain αAI-1.

We have developed a conceptual model describing a pathway on how the presence of αAI-1 in genetically modified (GM) legume seeds might negatively interfere with the biological control services provided by bruchid parasitoids[2]. Subsequently, we assessed the compatibility of GM seeds and bruchid parasitoids according to this model. We could show that parasitoids possess α-amylases susceptible to αAI-1[3], but exposure seems to be negligible and no adverse effects on the parasitoids could be detected.

The bruchid parasitoid *Heterospilus prosopidis* (Hymenoptera: Braconidae) searching for hosts hidden in chickpea seeds (Picture: G. Brändle, ART).

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[1] Lüthi C, Álvarez-Alfageme F, Ehlers JD, Higgins TJV, Romeis J. Resistance of αAI-1 transgenic chickpea (*Cicer arietinum*) and cowpea (*Vigna unguiculata*) dry grains to bruchid beetles (Coleoptera: Chrysomelidae). ***Bulletin of Entomological Research***: in press.

[2] Lüthi C, Álvarez-Alfageme F, Romeis J 2012. The potential of transgenic legumes in integrated bruchid management: Assessing the impact on bruchid parasitoids. ***Julius-Kühn-Archiv*** 425: 979-984.

[3] Álvarez-Alfageme F, Lüthi C, Romeis J 2012. Characterization of digestive enzymes of bruchid parasitoids - initial steps for environmental risk assessment of genetically modified legumes. ***PLOS ONE*** 7: e36862.

Local induction of chemical defences in maize: spatio-temporal aspects of benzoxazinone metabolism

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Benzoxazinoid derivatives (BXDs) are the most abundant defensive secondary metabolites in many grasses. Recently, it has been shown that some BXDs are strongly inducible upon herbivory: while 2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-Glc) is the most abundant BXD in non-attacked maize seedlings, its methylated derivative 2-β-D-glucopyranosyloxy-4-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one (HDMBOA-Glc) becomes the major compound after herbivore challenge[1]. Although induction patterns have been well described at the whole plant level, much less is known about defence responses at smaller functional scales, e.g. within an attacked leaf. As small scale variation in BXD levels may influence herbivore foraging patterns, we characterised BXD induction across a single leaf. We correlated the induction patterns with defensive phytohormone abundance as well as the transcription of a putative O-methyl-transferase that converts DIMBOA-Glc to HDMBOA-Glc.

Spatially restricted feeding by *Spodoptera littoralis* caterpillars in the centre of maize leaves led to a strong accumulation of HDMBOA-Glc close to the feeding site after 24h of feeding. HDMBOA-Glc levels were also enhanced systemically towards the leaf tip, but not at the leaf-base. The increased biosynthesis of HDMBOA-Glc was preceded by a local jasmonate burst and the induction of the putative DIMBOA-Glc O-methyl-transferase. Both hormonal and transcriptional induction patterns closely mirrored the observed metabolic changes. Interestingly, in contrast to jasmonates and O-methyl-transferase transcripts, the concentration of HDMBOA-Glc increased even further once the caterpillars had been removed from the leaf. Based on these findings we hypothesise that the strong local induction of BXDs may force caterpillars to move around continually to avoid intoxication, thereby interrupting feeding activity and decreasing overall herbivore damage of the plant. Furthermore, the frequent displacement may increase the apparency of herbivores to their own natural enemies. Future work using BXD-mutants will shed light on the importance of local metabolic responses for plant defences in an ecophysiological context.

[1] Glauser G, Marti G, Villard N, Doyen GA, Wolfender JL, Turlings TCJ, Erb M 2011. Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. ***The Plant Journal*** 68: 901-911.

Anthropogenic disturbance as a driver of microspatial and microhabitat segregation in secondary contact zones of the *Centaurea stoebe* cytotypes

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In mixed ploidy populations, strong frequency-dependent mating will lead to the elimination of the less common cytotype unless prezygotic barriers enhance assortative mating. To-date, such barriers have only rarely been explored in mixed ploidy populations.

To determine the mechanisms involved in the coexistence of *Centaurea stoebe* (Asteraceae) cytotypes and their interactions at a small spatial scale, we investigated: 1) microspatial and microhabitat distributions, 2) competitive, life history and fitness traits, 3) flowering phenology and 4) gene flow (using cpDNA and microsatellites) in 5 natural sites of co-occurring diploid monocarpic and closely related polycarpic allotetraploid derivate. In addition, we evaluated changes in frequency of both cytotypes in an artificially created mixed-ploidy population and assessed the response of cytotypes to water stress in a greenhouse experiment.

In natural mixed ploidy populations, the cytotypes were spatially segregated at all sites with tetraploids colonizing drier microhabitats created by human-induced disturbances. Conversely, the tetraploid cytotypes were rare in more natural sites and sites with denser vegetation despite their superior persistence (polycarpic life cycle) and competitive ability (increased number of shoots). The seed set of tetraploid plants was strongly influenced by their frequency in agreement with minority cytotype exclusion hypothesis. Triploid hybrids were extremely rare and almost completely sterile, indicating a strong postzygotic barrier between cytotypes. In the artificial mixed ploidy population, we observed a strong decrease in the number of diploid plants over a period of four years. The cytotypes did not respond differentially to water stress treatments in the greenhouse experiment.

Our findings suggest that tetraploids immigrated later into diploid populations, and anthropogenic activities that created open niches and mediated propagule introductions were the major factors shaping the non-random distribution of cytotypes. Establishment of tetraploids was further facilitated by their superior persistence due to their perennial life cycle and repeated seed production. Our results highlight the importance of non-adaptive spatio-temporal processes in explaining microhabitat and microspatial segregation of cytotypes.

Climatic niche shifts are rare among terrestrial plant invaders

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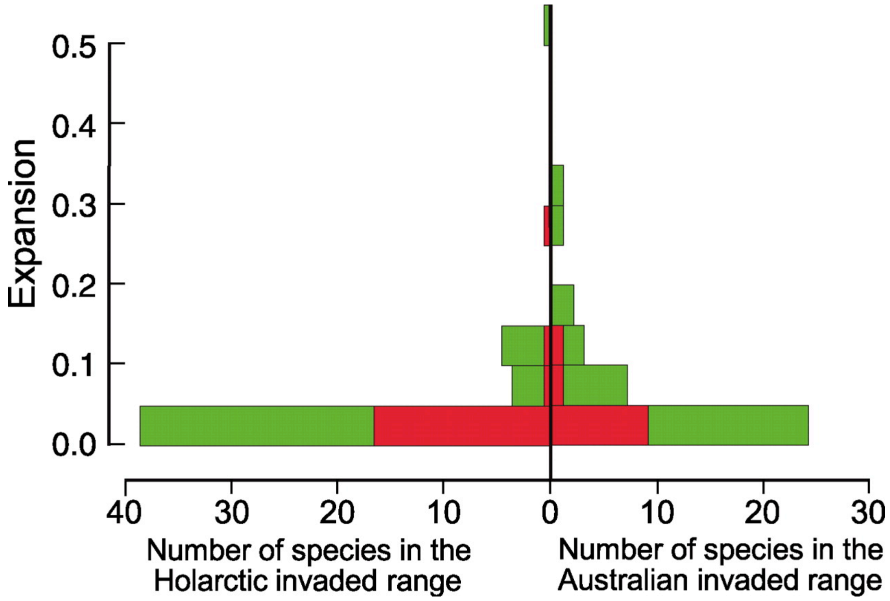
Biodiversity is put at threat by global change. Ecological niche modelling (ENM) is widely used to predict or to assess the effects of global change through predictions of potential species distribution under different climate or invasive species range. However, ENM relies on the pivotal assumption of niche conservatism (i.e. species ecological niche is conserved through space or time). This assumption has been challenged recently by evidence of niche shifts between native and invaded range of some invasive species. Here, we report the first large-scale test of niche conservatism for 50 terrestrial plant invaders between Eurasia (EU), North America (NA), and Australia (AU). We show that when analogue climates are compared between regions, fewer than 15% of species have more than 10% of their invaded distribution outside their native climatic niche. These findings reveal that substantial niche shifts are rare in terrestrial plant invaders, providing support for an appropriate use of ecological niche models for the prediction of both biological invasions and responses to climate change.



Expansion index is analogous to the proportion of the invasive distribution in climate that is new to the species but available in the native range. NA and EU species origins are shown in red and green, respectively.

Beneficial effects of *Pseudomonas putida* KT2440 on maize plants

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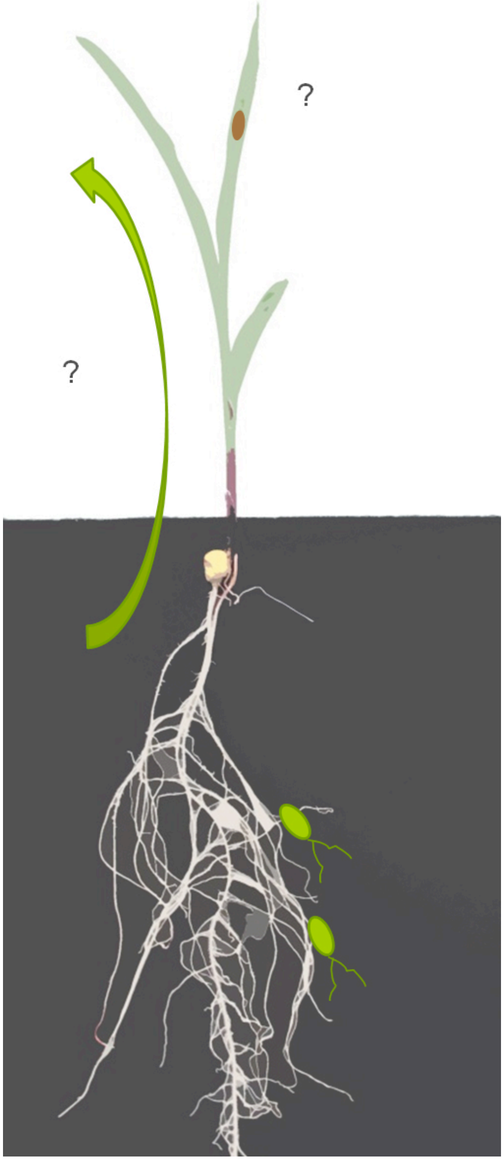
Beneficial interactions between root microorganisms and plants are common in nature. These non-pathogenic root-colonizing bacteria or fungi can help plant health via different mechanisms. Despite the economic importance of maize, studies about its interactions with beneficial rhizobacteria, such as induced systemic resistance, are less common than studies involving dicotyledonous plants.

The goal of the present study consists in a better understanding of the mechanisms that lead to defense reactions induced by beneficial bacteria in maize. For this purpose, various parameters affected by *Pseudomonas putida* KT2440 have been tested in maize plants. Root inoculation of maize seedlings with these bacteria induced defense reactions in leaves against the hemibiotrophic pathogen *Colletotrichum graminicola*, the causal agent of corn anthracnose, showing the potential of *P. putida* KT2440 to induce resistance in maize plants. Moreover, experiments have been conducted to test the effect of the presence of *P. putida* KT2440 on the leaf herbivory. Plants were infested with larvae of *Spodoptera littoralis*, a generalist herbivore, and *Spodoptera frugiperda*, a specialist herbivore. These results indicate that in addition to inducing resistance against *C. graminicola* the bacteria induce anti-herbivore defense in the plants. Interestingly, this defense is effective against the generalist but seems to be suppressed or coped with by the specialist.

To better understand these induced resistance mechanisms, further studies have been conducted to analyze the effect of the presence of *P. putida* KT2440 on plant roots on the plant defense gene expression and secondary metabolism.



Analysis of different beneficial effects induced by the presence of the rhizobacteria *Pseudomonas putida* KT2440 on maize plants and study of the mechanisms involved in this interaction.

Signal perception in phototropism

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Phototropism is the directed growth of plant organs towards a light source. For plants as sessile organisms, phototropism is extremely important, as it enables them to reach optimal amounts of light for photosynthesis and biomass production. Blue light that triggers phototropism is perceived by the phototropin (phot) photoreceptors. In *Arabidopsis*, there are two phototropins, phot1 and phot2. Both proteins play overlapping roles in the phototropic response to high light conditions while phot1 solely mediates the response to low-fluence light. Phototropic bending is achieved by asymmetrical cell growth in the elongation zone of the hypocotyl of etiolated seedlings. The current understanding of the mechanism behind phototropism suggests that the response is mediated by the following chain of events: (1) Incoming blue light is perceived by the phototropins which are light-activated kinases, (2) light perception triggers a signal transduction chain that (3) leads to the formation of a lateral auxin gradient, (4) auxin is perceived, and (5) triggers signaling networks that (6) control asymmetrical cell elongation and cell growth, ultimately leading to bending of the hypocotyl to align photosynthetic tissues with incoming light. While for coleoptiles of monocots it has been shown that signal perception takes place in the top 2 mm, there is no data so far for dicots like *Arabidopsis*. Using different genetic and physiological approaches we were able to show that the light signal leading to phototropic bending is perceived in or directly below the apical hook region, above the elongation zone. Thus, transport of auxin to this region and its subsequent perception are prerequisites for the establishment of the necessary auxin gradient and finally asymmetric growth.

In vivo recordings of action potential-like depolarizations in the phloem during insect feeding

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The balanced responses of plants to insect feeding are determined by a complex signalling network that links physiological responses to regulation of gene expression. Many players involved in the physiological responses that lead to plant defence remain unidentified, notably the membrane proteins that mediate ion fluxes, i.e. ion channels, pumps and/or transporters. In order to functionally identify the molecular effectors underlying wound-induced ion fluxes, we have applied the Electrical Penetration Graph (EPG, Fig. 1a), which is suitable for acquisition of membrane potential data, a parameter that is directly determined by the activity of ion flux-mediating proteins. With this approach, we were able to stably monitor the membrane potential in sieve tubes of *Arabidopsis* leaves during *ad libitum* caterpillar chewing. Mild feeding wounds triggered action potential-like depolarizations in the wounded leaves. These action potentials appeared to decrease in size with distance from the wound (Fig. 1b), and were not transmitted to unwounded connected leaves. In contrast, more severe wounds that totally or partially sectioned the midrib elicited large and complex depolarizations in unwounded connected leaves. These results suggest that plants have evolved different ion channel sets to respond to different wound types.

Currently, we are quantitatively characterizing the biophysical phenotype of electrophysiological responses in wild type *Arabidopsis*, as well as in knock out mutants for ion channels and pumps. Identifying the molecular basis for the wound-induced electrical potentials is the first step to understanding their eco-physiological significance.

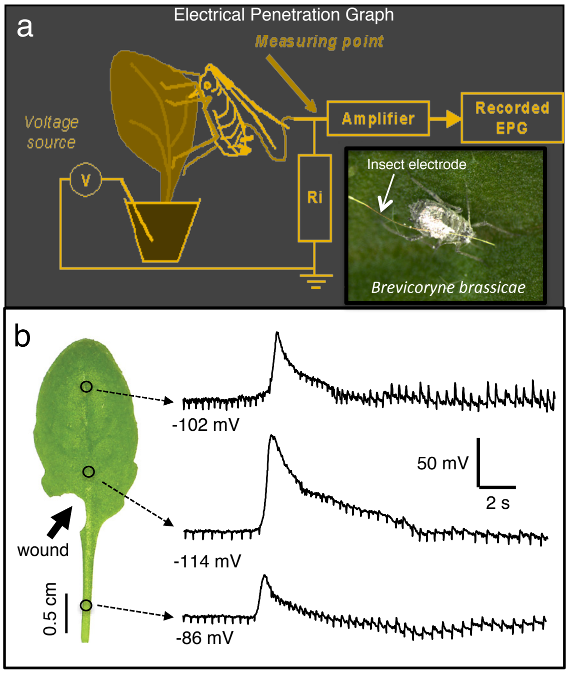

Figure 1. DC-EPG recordings of aphid feeding reveals action potential-like depolarizations in the phloem of herbivore-wounded *Arabidopsis* leaves.
a. The DC-EPG circuit. Bottom right: DC-EPG wired aphid. **b.** Action potential-like depolarizations recorded during caterpillar feeding (*Pieris brassicae*), at various distances from the feeding wound.

The role of Photosystem II Core Phosphatase in light acclimation in *Arabidopsis*

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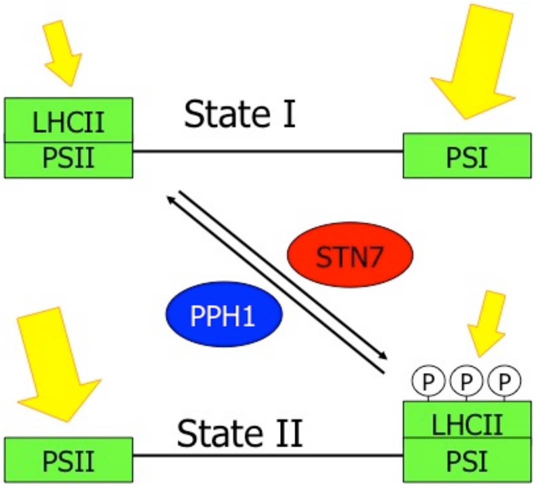
Thylakoid protein phosphorylation/de-phosphorylation reactions play a major role in the rapid acclimation of the photosynthetic apparatus to changes in light. Two paralogous kinases phosphorylate subsets of thylakoid membrane proteins. STN7 phosphorylates LHCII, the light harvesting antenna of photosystem II (PSII), to balance the activity of the two photosystems through state transitions. STN8, which is mainly involved in phosphorylation of PSII, influences folding of the thylakoid membranes and repair of PSII after photo-damage. The rapid reversibility of these acclimatory responses requires the action of protein phosphatases.

In a reverse genetic screen we have identified the chloroplast PP2C phosphatase, PBCP (PHOTOSYSTEM II CORE PHOSPHATASE), which is required for efficient de-phosphorylation of PSII. Its targets identified by immunoblotting and mass spectrometry largely coincide with those of the kinase STN8. The recombinant phosphatase is active *in vitro* on a synthetic substrate or on isolated thylakoids. Thylakoid folding is affected in the absence of PBCP, while its over-expression alters the kinetics of state transitions. PBCP and STN8 form an antagonistic kinase and phosphatase pair whose substrate specificity and physiological function are distinct from those of STN7 and the counteracting phosphatase PPH1 (TAP38), but their activities may overlap to some degree.



State transitions: Plants acclimate to changes in light conditions through state transitions. They balance the differences in excitation energy between two photosystems, PSI and PSII, by dynamic allocation of the LHCII antenna.

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1 Samol I, Shapiguzov A, Ingelsson B, Fucile G, Crèvecoeur M, Vener AV, Rochaix J-D, and Goldschmidt-Clermont M 2012. Identification of a photosystem II phosphatase involved in light acclimation in *Arabidopsis*. ***Plant Cell*** 24: 2596-2609.

Evolutionary dynamics of retrotransposon in response to polyploidy

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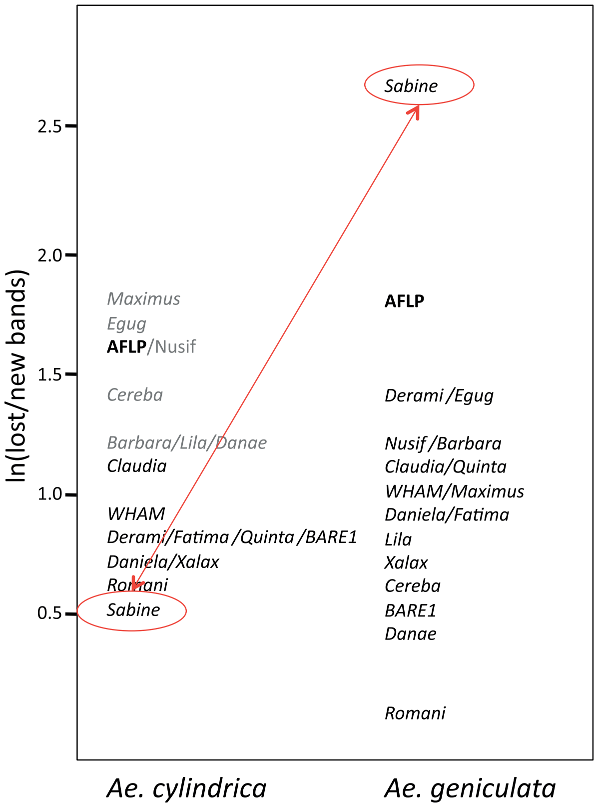
Retrotransposons are jumping genes representing the major fraction of plant genomes. The episodic activation of these intragenomic parasites plays a pivotal role in fuelling host genome reorganization and, ultimately, diversification. Allopolyploidy is a fascinating model to investigate genome evolution, because the merging of divergent genomes reveals conflicts among retrotransposons, leading to their activation. Using wild wheats as a model system, our goal is to better understand the evolutionary dynamics of major retrotransposon families and to assess genome restructuring in polyploids. As a first step, we developed an approach based on 454 low coverage genome sequencing (2.5%) to distinguish quiescent retrotransposon families from those that recently proliferated. Then, restructuring of selected retrotransposon fractions in tetraploids through molecular fingerprinting (AFLP, marking random sequences vs. SSAP, marking insertions of specific retrotransposons) was assessed, revealing retrotransposon families that strongly proliferated whereas others that mostly deleted. This study highlights a suitable approach to investigate the differential dynamics of retrotransposons in response to hybridization, allowing to address the evolutionary consequences of their activity on biological diversification.



Ratio of lost/new bands of 17 retrotransposons (SSAP) and random sequences (AFLP) in *Aegilops* allotetraploids as compared to diploid progenitors. Retrotransposons mentioned in black indicate significantly different ratio than random sequences, revealing specific evolutionary trajectories of retrotransposons (e.g. Sabine) after polyploidy.

Regulation of flavonol biosynthesis is an important determinant of *Petunia* flower colour and pollinator choice

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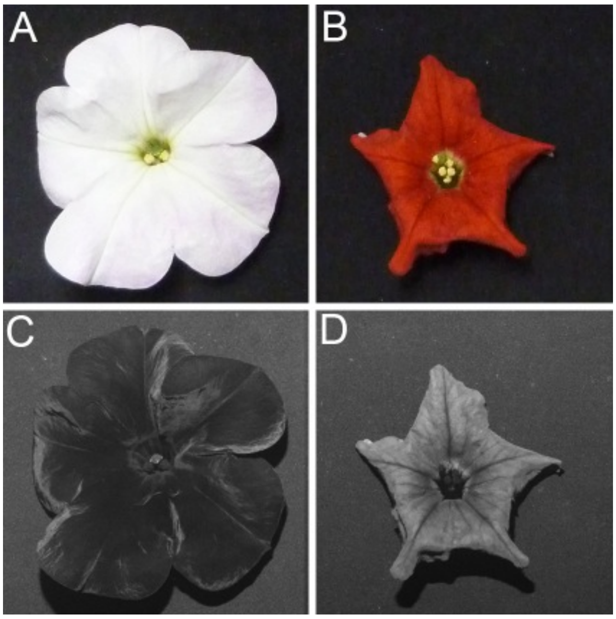
Floral traits determine which types of animal pollinators will visit and pollinate flowers. In the *Petunia* genus, closely related species are characterised by strikingly different floral traits, including visible and ultraviolet (UV) colour. *P. axillaris* has near-white flowers but produces a high level of flavonols which absorb UV light. *P. exserta* has red flowers due to the production of anthocyanin pigments and the flowers possess a comparatively low level of flavonols. We are interested in finding the key genes responsible for the difference in flavonol levels between these two species.

Quantitative trait loci (QTL) analyses of a *P. axillaris* and *P. exserta* F_2 population have identified genomic loci important for UV colour. A locus of major effect on ChrII segregates with *FLS*, the biosynthetic gene responsible for flavonol production. Analysis of introgression lines shows that this locus is distinct from *FLS* and that it is likely to be a regulator of *FLS*.

In *Arabidopsis*, *MYB11*, *MYB12* and *MYB111*, members of subgroup 7 of the family of R2R3-MYB transcription factors, are involved in the regulation of flavonol biosynthesis[1]. Homologues in Solanaceae also affect flavonol production. Using transcriptome data, a homologue of subgroup 7 has been found that underlies the QTL on ChrII and shows differential expression between *P. axillaris* and *P. exserta*. The current work is focused on verifying whether this gene plays a role in the regulation of flavonol biosynthesis in *Petunia*. Additionally, a transposon tagging approach is being undertaken to knock-out this putative regulator.



Petunia axillaris axillaris N (A, C) and *P. exserta* (B, D) absorb differently in visible light (A, B) and ultraviolet light (C, D).

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[1] Stracke R, Ishihara H, Huep G, Barsch A, Mehrtens F, Niehaus K, Weisshaar B 2007. Differential regulation of closely related R2R3-MYB transcription factors controls flavonol accumulation in different parts of the *Arabidopsis thaliana* seedling. ***The Plant Journal*** 50: 660-677.

Nuclear beta-amylases modulating brassinosteroid responses

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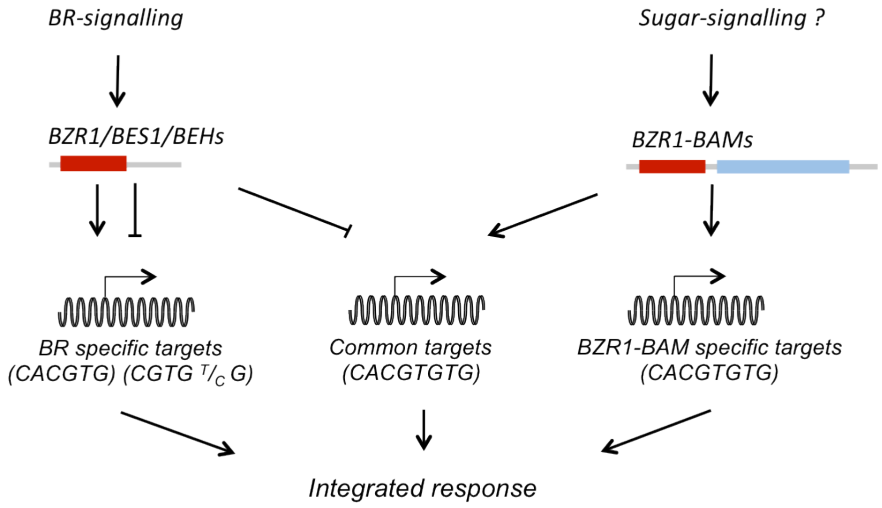
Plant architecture is controlled by multiple environmental and endogenous clues, including hormones, light, temperature, and the availability of carbohydrates. Most of these factors are sensed and reported by distinct signalling pathways, which have to converge into an integrated response.

Our group identified two β-amylases (BAMs) in *Arabidopsis* as potential integrators of sugar and brassinosteroid signalling events. Usually BAMs are enzymes which degrade starch to maltose in the chloroplast at night. In contrast, *BAM7* and *BAM8* (*BZR1-BAMs*) were shown to localise to the cell nucleus. Both isoforms contain beside their BAM domain a conserved DNA-binding domain of the BZR1-type, first described in the transcriptional regulators *BES1* (*bri1-EMS-supressor 1*) and *BZR1* (*BRASSINAZOLE RESISTANT 1*), which are involved in brassinosteroid (BR) signalling. The similarities of the DNA-binding domains and the bound *cis*-regulatory elements suggest a crosstalk of BZR1-BAMs with BR-signalling pathways. By genetic studies and reporter gene assays we demonstrate an antagonistic action of BZR-family transcription factors and BZR1-BAMs on the promoter region of common target genes. Co-immunoprecipitation coupled to mass-spectrometry identified 14-3-3 proteins as putative common interaction partners. Therefore, a well-equilibrated interplay of both transcription factor families is important for normal plant growth and development.



Working model connecting brassinosteroid signaling pathways with BZR1-BAM mediated signaling events.

Dissecting impact of plant invaders: do invaders behave differently in the new range?

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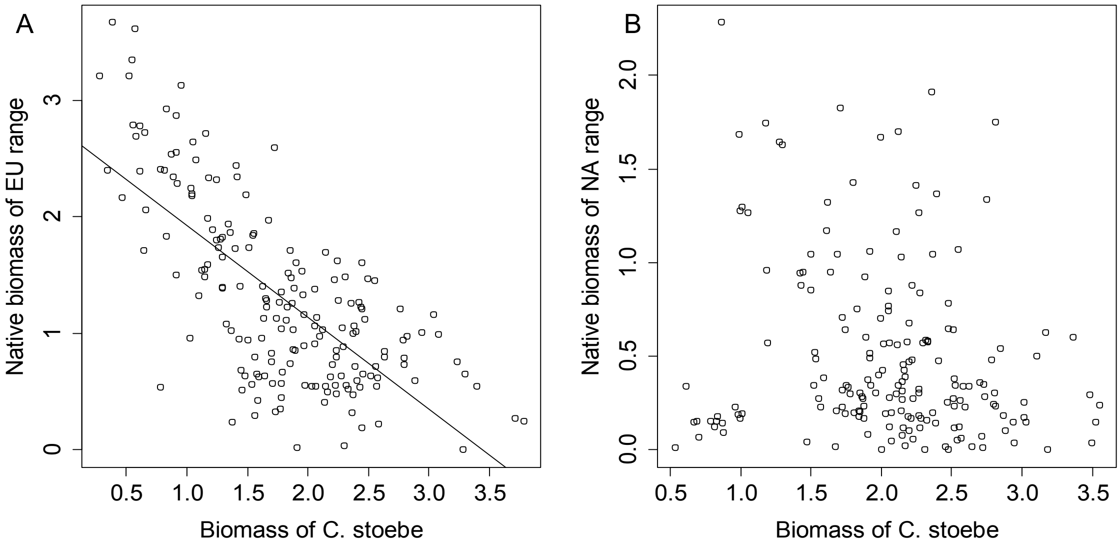
Impact of exotic plant invaders is hypothesized to be driven by either competition for the same limiting resources, or by unique traits of the invader. These processes might be context specific, explaining a differential impact in the native vs. introduced range. With the help of a conceptual framework, we aimed at identifying the relationship between invader biomass and impact in the invasive *Centaurea stoebe* by conducting pairwise competition experiments with 15 European (old) and 15 North American (new) neighbours. Old neighbours grew larger and could use available soil moisture more efficiently for growth than new neighbours. Interestingly, biomass of *C. stoebe* explained a substantial amount of the variation in biomass of the co-evolved neighbours, but not of the new "naïve" neighbours. Thus, at home impact seems to be driven by competition for the same limiting resources, while away by other factors, with important consequences for the management of this invader.



Relationship between the biomass (g) of *Centaurea stoebe* and that of European (A) and North American (B) neighbours in competition condition.

The relative impact of factors affecting species distribution prediction

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Species distribution models are increasingly used to address conservation questions. Their predictive capacity therefore requires careful evaluation. Previous studies have shown how individual factors used in model construction, such as sample size, sampling bias, modelling method, species characteristics or spatial autocorrelation, can affect prediction, for instance by contributing uncertainty to future projections. Clearly, reducing sample size or increasing sampling bias degrades prediction accuracy. However, the influence on predictions of each factor relative to other factors used in the model building process has never before been comprehensively assessed. As a result, the relative effect sizes of the different factors are largely unknown, despite some probably having negligible effects compared to others.

We propose to examine the relative importance of five key factors, using simulated data, for which truth is known. We account for stochasticity in species distribution and sampling pattern based on replications for each combination of all factors. We show that most of the variation in prediction accuracy is due to sample size. Counter to repeatedly reported concerns, spatial autocorrelation showed only small effects compared to other factors. These results shed important light on which factors need to be controlled when building species distribution models.

The influence of pollen chemical composition on bee-flower relationships

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The relationships between bees and flowers are complex and not merely mutualistic, but are better described as a reciprocal exploitation. Obviously, the primary interest of the bees is not to pollinate the flowers, but to efficiently collect pollen and nectar, their main larval food source. While nectar is chiefly produced as a reward for pollinators, pollen is a valuable resource, rich in protein and thus expensive for the plant, and essential for its reproduction. Therefore, many flowers have developed well-documented morphological adaptations to avoid excessive pollen harvesting by bees. It remains largely unclear, however, whether plants protect their pollen through secondary chemistry, as they do for other tissues. In fact, the secondary chemical composition of pollen is highly complex and probably plays a dual role in attracting visitors and protecting the pollen. The goal of this project is to examine how pollen chemical composition influences, and is influenced by, interactions with pollinators. We are focusing on several plant families presenting strikingly different pollination modes: the *Gesneriaceae*, a *Neotropical* family with strongly different pollination syndromes (either hummingbirds or bees), and the *Boraginaceae*, highly visited by bees but with highly variable floral morphologies: in some species the anthers are free and the pollen entirely exposed, while in other the anthers are hidden in a narrow floral tube. Pollen chemical composition is studied by LC-MS analyses.

Hoplitis adunca on *Echium vulgare* (picture Andreas Müller)

We hypothesize that plants with easily accessible pollen may possess toxic secondary compounds to prevent excessive pollen losses to the bees; in contrast, we expect plants with concealed pollen to have lower contents in secondary compounds in the pollen. Our first results show that only few *Boraginaceae* species show high alkaloid concentration in the pollen, and that most of these have rather exposed pollen. In contrast, *Boraginaceae* concealing their pollen in narrow floral tubes tend to have lower alkaloid concentrations. In the *Gesneriaceae*, the secondary compounds composition differs between bird and bee pollinated flowers. Bioassays and further analyses will be performed to confirm the influence of the pyrrolizidine alkaloids on bees.

An induced volatile as a poisonous repellent: the curious case of indole

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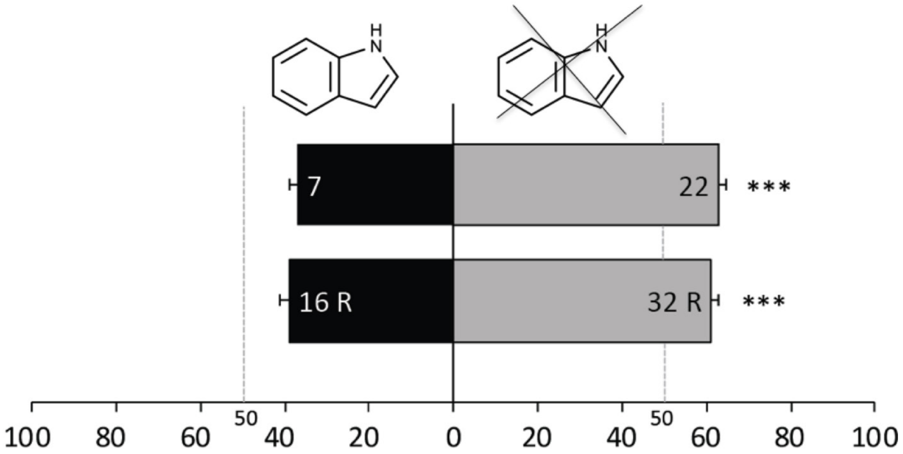
In response to insect attack, many plants release volatile indole. Yet, the function of this compound in plant-insect interactions has remained unclear. In maize, indole is a shared precursor for the biosynthesis of the essential amino acid tryptophane, but also for the toxic benzoxazinoids, and its release is regulated in an herbivore specific manner by the indole-3-glycerol phosphate lyase IGL. We found that indole reduces herbivore damage in maize in the field, and in the laboratory repelled larvae, as well as adults of the generalist lepidopteran herbivore *Spodoptera littoralis*. Phytochemical profiling revealed that a fraction of the IGL-dependent induced indole is not released into the headspace, but incorporated into benzoxazinoids and tryptophane. Suprisingly *S. littoralis* grew better on indole-producing maize plants than on IGL-knock-outs. This effect was dependent on the direct exposure of the herbivore to the volatile and was observed also on artificial diet. However, these growth effects were not caused by an increase in food consumption, but by induced physiological changes in the insects that ultimately reduced its survival and overall fitness. This new defensive mode of action of a plant volatile provides a direct adaptive context for the evolution of induced volatiles in plants.



Spodoptera littoralis larvae are repelled by volatile indole. In a four-arm olfactometer, larvae were given a choice between odour sources from indole-producing maize plants (7 and 16R) and IGL-knock-out maize plants (22 and 32R).

Loss of self-incompatibility in the toadflax *Linaria cavanillesii*: causes and consequences

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Flowers display an amazing variety of forms, shapes and colours. One striking contrast is between flowers that are obligately outcrossers and those that can self-fertilize. Evolution toward selfing is one of the most common transitions in flowering plants, but its causes, mechanisms and consequences continue to be the focus of much interest. *Linaria cavanillesii* (Scrophulariaceae) is a toadfloax species endemic to south-eastern Spain. This insect-pollinated species exhibits variation in its mating system, with most populations possessing functional gametophytic self-incompatibility (SI), but with northern populations having lost SI (being self-compatible, SC). Typically, the loss of SI leads to the evolution of a 'selfing syndrome', with smaller flowers that produce less nectar, and lower pollen:ovule ratios. In contrast, there are no obvious morphological differences in flowers between SI and SC populations. The aim of this project is to determine what might have caused transition between SI and SC in this species, to ascertain its effect on the mating system and pollination biology, and to characterize its population genetic consequences, both at the self-incompatibility locus, at fitness-determining loci, and broadly across the genome. We are particularly interested in how and why an outcrossing syndrome appears to have been maintained despite the loss of SI. The poster will summarize the background and aims of the project and the approached that are being adopted.

Non-redundant control of rice arbuscular mycorrhizal symbiosis by two phosphate transporters

Yang S-Y, *Jakobsen I, Rentsch D, Hirochika H, Sundaresan V, Salamin N, Heuer S, Gheyselinck J and Paszkowski U*

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Phosphate (Pi) acquisition of crops via arbuscular mycorrhizal (AM) symbiosis gains increasing importance due to limited high-grade rock Pi reserves and demand for environmentally sustainable agriculture. We found that 70% of the overall Pi acquired by rice is delivered via the symbiotic route. To better understand this pathway we combined genetic, molecular and physiological approaches to determine the specific functions of two symbiosis-specific rice Pi transporters, PT11 and PT13. The PT11 lineage of proteins from mono- and dicotyledons is most closely related to Pi transporters from the ancient moss, indicating an early evolutionary origin. In contrast, PT13, arose in the Poaceae, suggesting that grasses have acquired a particular genetic redundancy to secure symbiotic Pi acquisition. Surprisingly, mutations in either PT11 or PT13 affected development of the symbiosis, demonstrating that both genes are essential for AM symbiosis. For symbiotic Pi uptake, however, only PT11 is necessary and sufficient. Consequently, our results demonstrate that mycorrhizal rice depends on the AM symbiosis to satisfy its Pi demands, which is mediated by a single functional Pi transporter, PT11.

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