

SwissPLANT 2020



Symposium Plant Science Research in Switzerland

swissplantscienceweb.ch – 29th edition

29 – 31 January 2020
Ovronnaz, Switzerland



SwissPLANT 2020

Swiss Plant Symposium 2020 29–31 January 2020 Ovronnaz, Switzerland

Table of content

2	Welcome by the SPSW president
3	Symposium program
7	Talks (abstracts in alphabetical order by presenting author)
39	Posters (abstracts in alphabetical order by last name)
46	List of participants
48	Impressum/Imprint

The Swiss Plant Science Web gratefully acknowledges the financial support for the SwissPlant 2020 conference by the listed sponsor:



Venue

Hôtel des bains d'Ovronnaz, 1911 Ovronnaz, Valais, Switzerland

Scientific Program Committee

Heinz Müller-Schärer, Markus Geisler, Ora Hazak, Felix Mauch, Laurent Mène-Saffrané,
Didier Reinhardt, Laure Weisskopf
University of Fribourg

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SwissPLANT 2020 – Welcome by the president

This year's SwissPLANT Symposium represents the 10th annual meeting of the plant science research community in Switzerland. It can also be seen as the 29th edition, counting from its inception as the "Swiss Plant Molecular and Cell Biology Conference" in 1992.

With the creation of the Swiss Plant Science Web in 2009, the scope of the conference was expanded to encompass all basic research in plant science. The first meeting in this new format was held in Meiringen 2011, and it proved to be a success. This year, on the initiative of the organizers from the University of Fribourg, we meet – for the first time! – in Ovronnaz (canton of Valais) to highlight and discuss current advances in plant science research.

The main aim of the symposium is to present research on form, function, genetics, ecology, and evolution of plants. We seek an integrative approach in order to increase the understanding of the complexity and diversity of the green world. We want to spread the word that plant science is central to addressing many of the key global issues of humanity in the 21st century.

I welcome all new Swiss Plant Science Web members. Your input and ideas will shape the long-term continuity of plant science research in Switzerland. The "SwissPLANT meeting" is the ideal place to start discussions on new collaborations.

The program was put together by our colleagues from the University of Fribourg who volunteered to serve on the 2020 committee. I thank Heinz Müller-Schärer, Markus Geisler, Ora Hazak, Felix Mauch, Laurent Mène-Saffrané, Didier Reinhardt and Laure Weisskopf for setting up a great program. Furthermore, I am very grateful that Sylvia Martínez (University of Basel), our SPSW coordinator, makes sure that everything runs smoothly.

Enjoy Swiss Plant Science in Ovronnaz – and the leisure moments, perhaps on the slopes or in the spectacular outdoor thermal pools ...

Thomas Boller, SPSW president

The Swiss Plant Science Web is the umbrella organization for plant science research and education at universities in Switzerland

Program

Wednesday, 29 January 2020

15:30 Swiss Plant Science Web strategic meeting for SPSW members

16:45 Welcome drink

17:10 Welcome by Thomas Boller, SPSW chair

17:15 Opening remarks by the program committee

Session I: Heinz Müller-Schärer (chair)

17:20 Makus Geisler | U Fribourg
A conserved D/E-P motif defines the auxin transport capacity of ABCB-type auxin transporters

17:40 Sebastian Soyk | U Lausanne
Cryptic genetic variation with impact on crop productivity

18:00 Christian Parisod | U Bern
Allopolyploid radiation despite eco-genetic additivity of diploids

18:40 Dinner

Session II: Markus Geisler (chair)

20:20 Michael Hothorn | U Geneva
Investigating inorganic polyphosphate metabolism in plants – how to get yourself in and out of trouble

20:40 Kentaro Shimizu | U Zurich
Machine learning of transcriptome data in natura: Drought as a trigger for flowering in aseasonal tropics

21:00 Christiane Nawrath | U Lausanne
The role of AtABCG32 transporters in cutin formation

21:20 Felix Kessler | U Neuchâtel
Proton Gradient Regulation 6 (PGR6) maintains plastoquinone levels in the photosynthetic electron transport chain

21:40 Get-together at the hotel bar

Thursday, 30 January 2020

07:00	Breakfast begins
	Session III: Ora Hazak (chair)
08:00	<u>Heinz Müller-Schärer</u> U Fribourg How to better predict long-term benefits and risks in weed biocontrol: An evolutionary perspective
08:20	<u>Yan Sun</u> U Fribourg Rapid evolution of a plant invader in response to biological control and global warming
08:40	<u>Iga Tomczynska</u> U Fribourg A pathogen effector protein promotes symplastic cell-to-cell trafficking by physical interaction with plasmodesmata-localized callose synthases
09:00	<u>Elisabeth Truernit</u> ETH Zurich An update on the role of OCTOPUS-LIKE genes in root development
09:20	<u>Niko Geldner</u> U Lausanne Root damage and immune responses at cellular resolution
09:40	Coffee Break
	Session IV: Laurent Mène-Saffrané (chair)
10:10	<u>Daniel Croll</u> U Neuchâtel Parasites within parasites: Selfish elements as drivers of interactions between pathogens and plants
10:30	<u>Emilie Demarsy</u> U Geneva A bi-phasic model of chloroplast biogenesis during de-etiolation of <i>Arabidopsis thaliana</i>
10:50	<u>Christian Fankhauser</u> U Lausanne Reaching out for the sun: Dealing with the threat of carbon deprivation
11:10	<u>Pauline Jullien</u> U Bern <i>Arabidopsis</i> ARGONAUTE3 function upon induction by the bacterial virulence factor SyringolinA
11:30	<u>Stefan Grob</u> U Zurich Transgene Silencing in 3D – how a chromosomal KNOT can inactivate foreign DNA elements
11:50	Leisure time (Lunch on your own, skiing, snowshoeing, swimming...)

	Session V: Felix Mauch (chair)
17:20	<u>Cyril Zipfel</u> U Zurich Regulation of plant immunity by phytochemicals
17:40	<u>Spoorthi Kalleda</u> U Zurich A green toxin: Plant's chlorophyll/chlorophyllase-mediated binary defense against chewing herbivores
18:00	<u>Roman Ulm</u> U Geneva UV-B photoreceptor signalling and responses
18:20	<u>Christian Körner</u> U Basel Plant water relations re-visited
18:30	Dinner
20:20	Poster Session (drinks will be served)

Friday, 31 January 2020

- 07:00 Breakfast begins
- Session VI: Didier Reinhardt (chair)**
- 08:00 Julia Santiago | U Lausanne
Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth
- 08:20 Paolo Longoni | U Neuchâtel
Role of the major Light Harvesting Complex II under fluctuating light
- 08:40 Ricardo Machado | U Bern
Engineering bacterial symbionts of nematodes improves biocontrol potential of the western corn rootworm
- 09:00 Xavier Perret | U Geneva
Lotus corniculatus hosts a surprisingly large diversity of nitrogen-fixing rhizobia in Switzerland
- 09:20 Barbara Pfister | ETH Zurich
From starch biosynthesis to chloroplast development – neofunctionalization of BRANCHING ENZYME1 (BE1)
- 09:40 Coffee break
- Session VII: Laure Weisskopf (chair)**
- 10:10 Didier Reinhardt | U Fribourg
Nutrient exchange in arbuscular mycorrhiza: Carbon supply from host to fungus
- 10:30 Pierre Goloubinoff | U Fribourg
Is Hsp70 recruited to re-potentiate heat-depolarized calcium channels in the plasma membrane of plants?
- 10:50 Christoph Ringli | U Zurich
Analysis of the LRX-RALF-FER signaling network involved in cell growth and cell wall integrity sensing
- 11:10 Bruno Studer | ETH Zurich
Genomics-based approaches to characterise and efficiently utilize genetic diversity for plant breeding
- 11:30 Bernhard Schmid | U Zurich
Standing epigenetic variation speeds up plant adaptation
- 11:50 Closing remarks

TALKS

Abstracts

Parasites within parasites: Selfish elements as drivers of interactions between pathogens and plants

Daniel Croll

Laboratory of Evolutionary Genetics, Institute of
Biology, University of Neuchâtel

Transposable elements are core constituents of eukaryotic genomes including the genomes of most plant pathogens. Transposable elements are intricately associated with pathogenicity, because transposable elements and pathogenicity genes often share similar genomic locations. Transposable elements also generated key adaptive genetic variation in pathogen populations. Hence, transposable elements and their plant pathogen host genomes are on complex evolutionary trajectories. To resolve these trajectories, we use large population genomic datasets of the major wheat pathogen *Zymoseptoria tritici*. First, we analyze co-regulation of transposable elements and pathogenicity genes over the course of a plant infection. We find that stress imposed by host immunity induces de-repression of transposable elements. However, the synchronicity with pathogenicity genes depends on the nature of the transposable element and the genetic background of the pathogen genome. Then, we analyze complete genomes of isolates spanning the worldwide distribution and show that transposable element activity explains a significant fraction of all intra-specific structural variation. We find that the demographic history of the pathogen had an impact on transposable element control contributing to extant population differentiation. In conjunction, our results show that transposable elements impose strong trade-offs on plant pathogen evolution by reshaping the genome structure and promoting adaptation.

CDEFGHIJKLMNOPQRSTUVWXYZ

A bi-phasic model of chloroplast biogenesis during de-etiolation of *Arabidopsis thaliana*

Rosa Pipitone¹, Simona Eicke², Barbara Pfister², Gaetan
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Sam Zeeman², Felix Kessler¹, **Emilie Demarsy**^{1,5}

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of Geneva

Light triggers chloroplast differentiation in dark grown (etiolated) seedlings. A precursor organelle, the etioplast, transforms into a photosynthesizing chloroplast. Over the course of just a few hours, an extensive photosynthetic membrane system, the thylakoid, emerges. This requires synthesis of highly abundant membrane lipids as well as specific photosynthesis-associated proteins. But the sequence of events during chloroplast differentiation is still unclear. Using Serial Block Face Scanning Microscopy (SBF-SEM) we generated a time course of 3D reconstructions of entire cells and chloroplasts during differentiation, revealing number, volume as well as envelope and thylakoid membrane surface. The (ultra)structural data are complete with quantitative lipid and proteome data that together provide a time-resolved, multi-dimensional analysis of chloroplast differentiation. The superimposition of the structural and biochemical data reveals two distinct phases of chloroplast biogenesis; an initial "Structure Establishment Phase"

enabling onset of photosynthesis, followed by a "Chloroplast Proliferation Phase" coinciding with cell expansion. We present a mathematical model describing the expansion of the thylakoid membrane area during chloroplast differentiation. Thereby we establish a roadmap to chloroplast differentiation, a critical process towards photoautotrophic growth and survival of young plants.

ABC D

Reaching out for the sun: Dealing with the threat of carbon deprivation

Christian Fankhauser, Olivier Michaud, Mieke de Wit, Anne-Sophie Fiorucci and Yetkin Çaka Ince

Center for Integrative Genomics, Faculty of Biology and Medicine, University of Lausanne

Light is a vital resource for plants, which compete for its availability particularly in dense communities, which are typical in agriculture. Plants possess multiple photosensory receptors to detect the presence of competitors and thereby adjust their growth and developmental strategies accordingly. I will discuss the photoperception mechanisms and growth responses elicited by the neighboring vegetation in *Arabidopsis* and *Brassica rapa*, two typical shade-avoiding species. These responses include rapid shade-induced organ-specific transcriptional reprogramming mediated by Phytochrome Interacting Factors (PIFs) (1). In young seedlings shade leads to a rapid burst of auxin production in cotyledons that is then transported to the hypocotyl where it promotes elongation. Later in development similar regulatory mechanisms underlie leaf repositioning that is restricted to the shaded part of the plant (2). I will present evidence for rapid reallocation of carbon from the main photosynthetic source tissues towards elongating stems. This resource reallocation is essential for rapid growth elicited by neighbor threat (3). Finally, I will discuss our latest unpublished data regarding the utilization of new resources to fuel growth in the stem with an emphasis on lipid metabolism.

- (1) Kohnen, M.V., et al., Neighbor Detection Induces Organ-Specific Transcriptomes, Revealing Patterns Underlying Hypocotyl-Specific Growth. *Plant Cell*, 2016. 28:2889-2904.
- (2) Michaud, O., et al., Local auxin production underlies a spatially restricted neighbor-detection response in *Arabidopsis*. *PNAS*, 2017. 114:7444-744
- (3) de Wit, M., et al., Changes in resource partitioning between and within organs support growth adjustment to neighbor proximity in Brassicaceae seedlings. *PNAS*, 2018. 115:E9953-E9961.

A conserved D/E-P motif defines the auxin transport capacity of ABCB-type auxin transporters

Markus Geisler

University of Fribourg, Department of Biology

Auxin transport activity of ABCB-type auxin transporters was suggested to be regulated by physical interaction with the *bona fide* peptidylprolyl *cis-trans* isomerase (PPIase), TWISTED DWARF1, but all attempts to demonstrate such an activity on TWISTED DWARF1 have failed so far. By using a structure-based approach we have identified a series of surface-exposed proline residues in the C-terminal nucleotide binding fold of *Arabidopsis* ABCB1 that do not alter ABCB1 protein stability or location but its catalytic transport activity. P1.008 was uncovered as part of a signature D/E-P motif that seems to be specific for Auxin-Transporting ABCBs, we now refer to as ATAs. Beside the proline, also mutation of the acidic moiety prior to the proline abolishes auxin transport activity by ABCB1. So far, all higher plant ABCBs for that auxin transport was safely diagnosed carry this conserved motif underlining its diagnostic potential. Introduction of this D/E-P motif into malate importer, ABCB14, increases its malate import but also its background auxin transport activity, suggesting that this motif has an impact on transport activity in general. The D/E-P1.008 motif is also important but not essential for interaction between ABCB1 and TWISTED DWARF1 supporting a previously suggested scenario in that TWD1 acts as a positive modulator of ABCB transport activity by means of its PPIase activity.

Root damage and immune responses at cellular resolution

Niko Geldner

University of Lausanne

Microbe-associated molecular pattern (MAMP) recognition is crucial to the plant's immune system, but how this sophisticated perception system can be usefully deployed in roots, continuously exposed to bacteria, remains unresolved. We have analyzed MAMP receptor expression and responses at cellular resolution in *Arabidopsis* and found that differentiated outer layers, exposed to bacteria, show low receptor levels and lack MAMP responsiveness. However, these cells can be locally "gated" to become responsive, by either neighbor cell damage or emerging lateral roots. Laser-induced localized damage also leads to immune responses to an otherwise non-immunogenic, beneficial bacterium and enhances responses to a root pathogenic bacterium. Moreover, we find that single cell damage in roots leads to regional ROS and calcium waves, ethylene responses, but no detectable jasmonate responses. Treatment with DAMPs alone do not re-iterate laser-induced damage and, surprisingly, the very local upregulation of MAMP responses by damage is independent of ethylene signalling. Our findings demonstrate that spatially restricted receptor expression is crucial for an appropriate MAMP response in roots and helps to conceptualize how MAMP perception can be used despite a continuous presence of microbial patterns in the soil.

GHIJKLMNOPQRSTUVWXYZ

Is Hsp70 recruited to re-potentiate heat-depolarized calcium channels in the plasma membrane of plants?

Anthony Guihur and Pierre Goloubinoff

Dept of Plant Molecular Biology, Lausanne University

Specific cyclic nucleotide gated channels (CNGCs) in the plasma membrane of higher plants act as thermo-sensors (1). The land plant CNGC2 and CNGC4, which likely form hetero-tetramers, contain C-terminal cytosolic domains that can bind calmodulins and cyclic nucleotides. Upon temperature increase, the tensed closed CNGC channels readily open to mediate the transient entry in the cytosol of extracellular Ca^{2+} ions that can activate the bound calmodulin(s) and initiate a signalling cascade to produce heat-shock proteins (HSPs). Many HSPs act as molecular chaperones that protect proteins from heat-damages. Yet, within minutes, the relaxed, heat-depolarized calcium channels become hermetically closed again, stopping signalling for additional HSP production, despite the ongoing heat-stress. Up to five hours at low temperature are than needed for the heat-depolarized CNGCs to become re-potentiated and effectively produce a second, fully potent heat-shock response. Using the C-terminal cytosolic domain of CNGC2 as bait, we identified in a yeast-two-hybrid screen a unique plant DNAJC proteins. DNAJ co-chaperones generally target HSP70 onto protein complexes in need to be converted into structurally different complexes by the chaperone. Like Hsp60s, HSP70s can inject energy from ATP hydrolysis to convey, even under non-equilibrium conditions, stable misfolded proteins up a free energy landscape, and transiently convert them into a less stable native state (2). We thus hypothesized here that at non-HS temperatures this specific DNAJC is recruiting HSP70 onto the heat-depolarized

relaxed closed CNGCs to re-potentiate them into a different closed state with a higher free energy, poised to readily respond to a second heat-shock. We now investigate the mechanism by which these plant channels may become tensed again by the combined action of the DNAJC and HSP70. Having shot a first arrow, Wilhem Tell's muscles also needed to inject the energy of ATP hydrolysis to tense again his cross bow, and shoot a second arrow.

(1) Finka, A., Cuendet, A. F. H., Maathuis, F. J. M., Saidi, Y. and Goloubinoff, P. Plasma Membrane Cyclic Nucleotide Gated Calcium Channels Control Land Plant Thermal Sensing and Acquired Thermotolerance. *Plant Cell* 2012, 24,8, 3333-48
(2) De Los Rios, P. and P. Goloubinoff, Hsp70 chaperones use ATP to remodel native protein oligomers and stable aggregates by entropic pulling. *Nature Structural & Molecular Biology*, 2016. 23(9): p. 766-769

ABCDEF G

Transgene Silencing in 3D – How a Chromosomal *KNOT* Can Inactivate Foreign DNA Elements

Stefan Grob and Ueli Grossniklaus

Dept of Plant and Microbial Biology, University of Zurich

Cells require elaborate mechanisms to efficiently pack chromosomes in the nucleus, while still allowing access to the genetic information. In addition, three-dimensional (3D) chromosome architecture is linked to epigenetic processes and transcriptional activity. Despite progress in the field, well-established cases of functional relationships between transcription and 3D chromatin architecture remain rare. We previously identified a 3D chromatin structure in *Arabidopsis* termed the *KNOT*, in which ten genomic regions (*KEEs*) physically contact each other.

Here we show that *KEEs* are involved in the silencing of transgenes. Transgenes integrated in the genome can fold towards the *KNOT*, coinciding with their transcriptional silencing. Thus, transgene integration can lead to significant perturbation of 3D chromosome architecture. Regions adjacent to the insertion sites are not subjected to silencing, despite their dislocation within the nucleus. This novel silencing mechanism, termed *KNOT*-linked Silencing (KLS) may act independently of previously described silencing mechanisms, as we cannot observe any significant contribution of small RNAs and DNA methylation. KLS is heritable across generation and shows trans-silencing effects, as the introduction of *KNOT*-silenced transgenes can lead to the silencing of previously active transgenes.

GHIJKLMNOPQRSTUVWXYZ

Investigating inorganic polyphosphate metabolism in plants – how to get yourself in and out of trouble

Michael Hothorn

Structural Plant Biology Laboratory, Department of Botany and Plant Biology, University of Geneva

Inorganic polyphosphates (polyPs) are linear chains of orthophosphate units linked by phosphoanhydride bonds. They represent important stores of phosphate and energy in bacteria and in yeast cells, and are present in our red blood cells and bone marrow. Little is known however about polyPs in plants, their metabolism and their cellular functions. I will summarize our efforts to characterize polyPs in plants, from their detection in cells and tissues to the identification of polyP metabolizing enzymes and specific binding proteins. Notably, the characterization of the polyPase AtTTM3 led us to the discovery of a conserved bicistronic transcript, in which the polyP metabolizing enzyme is transcribed and translated together with a previously unknown cell cycle regulator essential for plant embryo development.

Catalytic core of a membrane-associated eukaryotic polyphosphate polymerase. Hothorn M, Neumann H, Lenherr ED, Wehner M, Rybin V, Hassa PO, Uttenweiler A, Reinhardt M, Schmidt A, Seiler J, Ladurner AG, Herrmann C, Scheffzek K, Mayer A. *Science*. 2009. 324(5926):513-6. doi: 10.1126/science.

References:

- Identity and functions of inorganic and inositol polyphosphates in plants. Lorenzo-Orts L, Couto D, Hothorn M. *New Phytol*. 2019. doi: 10.1111/nph.16129.
- Molecular characterization of CHAD domains as inorganic polyphosphate-binding modules. Lorenzo-Orts L, Hohmann U, Zhu J, Hothorn M. *Life Sci Alliance*. 2019. pii: e201900385. doi: 10.26508/lsa.201900385.
- Concerted expression of a cell cycle regulator and a metabolic enzyme from a bicistronic transcript in plants. Lorenzo-Orts L, Witthoef J, Deforges J, Martinez J, Loubéry S, Placzek A, Poirier Y, Hothorn LA, Jaillais Y, Hothorn M. *Nature Plants*. 2019. 5(2):184-193. doi: 10.1038/s41477-019-0358-3.
- Structural Determinants for Substrate Binding and Catalysis in Triphosphate Tunnel Metalloenzymes. Martinez J, Truffault V, Hothorn M. *J Biol Chem*. 2015. 290(38):23348-60. doi: 10.1074/jbc.M115.674473.

ABCDEFGHIH

***Arabidopsis* ARGONAUTE3 function upon induction by the bacterial virulence factor SyringolinA**

Diane MV Bonnet¹, Stefan Grob², Louis Tirot¹, Gregory Schott³ and **Pauline Jullien**¹

¹ Institute of Plant Sciences, University of Bern

² University of Zurich

³ ETH Zurich

Defense mechanisms against pathogens are key for organism's survival. In plant, small RNA pathways are an integrated part of the plant defense machinery against pathogens such as bacteria, viruses or fungi. We have recently shown that the AGO3 protein is induced upon infection by the bacteria *Pseudomonas*. This induction relies on the inhibition of the proteasome machinery by a bacterial virulence factor called SyringolinA. The induction of AGO3 expression is limited to the plant vasculature and more specifically in cells surrounding the plant vasculature. We could show that upon induction, AGO3 is required to allow a stronger induction of disease resistance genes. Intriguingly, this response is linked to the loading of transposon (TE) derived small RNA by AGO3. Reactivation of TE in the plant vasculature was shown to be induced by a bacterial flagellum protein called Flagellin. Flagellin activates the plant DNA demethylation pathway which in turn is required for the expression of defence genes necessary to prevent or reduce bacterial infection. We propose that AGO3 sequestration of TE small RNAs would prevent precocious re-methylation of TE and defence genes during infection.

J K L M N O P Q R S T U V W X Y Z

A green toxin: Plant's chlorophyll/chlorophyllase-mediated binary defense against chewing herbivores

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Chlorophyll (chl) is consumed as part of the daily diet of leaf-chewing insect herbivores. In *Arabidopsis thaliana*, chl and chlorophyllase (CLH) occur in different organelles of the intact cells. Upon cell disruption by larval feeding, CLH dephytylates chl to form chlorophyllide, that thwarts the larval growth. It remains elusive whether plants use chl/CLH as a primary anti-herbivore defense and if so, how insects overcome this binary defense.

We studied the chl/CLH defense in broccoli and rapeseed from the Brassicaceae, and, tomato, bell pepper and eggplant from the Solanaceae. Here, we show that *in vitro* CLH activity dramatically increased in damaged leaves of broccoli, rapeseed and bell pepper but not in tomato and eggplant. Similarly, simulated herbivory (applying *Spodoptera littoralis* larval regurgitant to freshly punctured wounds) resulted in chl upregulation and increased chlorophyllide levels, 2 h and 48 h after elicitation, respectively, compared to undamaged leaves in Brassicaceae but not in Solanaceae plants. Further, to unravel countermeasures evolved in chewing herbivores, we performed insect bioassays and metabolomics using *A. thaliana* chl mutants. Generalist *S. littoralis* and *S. exigua* larvae fed less and grew smaller on chl1-overexpression lines and showed higher chlorophyllide content in their midgut and

frass compared with larvae fed on wild-type and chl1-mutant lines. By contrast, specialist *P. brassicae* larvae grew similarly on these lines and did not show increased chlorophyllide levels in their tissues.

Next, we plan to evaluate crosstalk between jasmonate signaling and chl1 regulation in response to herbivory. These results will open new perspectives on the complex strategies of plants and insects adapting to the chl/CLH binary defense.

A B C D E F G H I J K

Proton Gradient Regulation 6 (PGR6) maintains plastoquinone levels in the photosynthetic electron transport chain

Felix Kessler

Institute of Biology, University of Neuchâtel

In addition to photosystems small molecules participate in photosynthetic electron transport. One of these is the membrane-soluble redox compound plastoquinone (PQ) (749.2 g/mol). In the electron transport chain PQ is reduced to plastoquinol (PQH₂) at Photosystem II, diffuses across the photosynthetic membrane and is re-oxidized at the cytochrome b6f complex. The proportion of PQ participating in photosynthetic electron transport is known as the photoactive PQ pool. But a large proportion of PQ does not participate in photosynthesis and is mostly stored in plastoglobules. Under highlight irradiation photoactive plastoquinone is destroyed. It must be replaced from the plastoglobule reservoir to maintain electron transport. PGR6 is an atypical kinase localized in plastoglobules. It lacks photoactive PQ under high light intensity. Under high light, electron transport is prematurely saturated in the *pgr6* mutant and Photosystem II is severely damaged. As a consequence of lacking photoactive PQ in *pgr6*, the important photoprotection mechanisms “non-photochemical quenching” and “state transitions” are strongly perturbed. The data demonstrate that *PGR6* is part of a mechanism that maintains adequate concentrations of PQ in the photosynthetic electron transport chain.

Pralon et al. COMMUNICATIONS BIOLOGY | (2019) 2:220 | <https://doi.org/10.1038/s42003-019-0477-4>

KLMNOPQRSTUVWXYZ

Plant water relations re-visited

Christian Körner

University of Basel

Plant water relations, particularly those of trees, have received increasing attention in the last decade, while over the 20-30 years before, only a small group of researchers worked in this field. For that reason, most university curricula lack courses on classical plant water relations, although this is perhaps the most central issue for plants since they established on land during the Devonian. In this presentation I will re-visit some basic paradigms of plant water relations and will recall common pitfalls and misconceptions. Among the most widespread misunderstandings are the function and interpretation of plant water potential, the term water use efficiency and its relation to carbon isotope signals, the role of stomata for CO₂ uptake and plant carbon relations under drought (dying from thirst or hunger?), and various aspects related to the hydraulic system of plants. In essence, I will challenge a number of traditional and more recent assumptions in this field of research. I will discuss unresolved issues and implications for functional plant ecology in the context of an increasing risk of drought in a warmer world.

ABCDEFGHIJK

Role of the major Light Harvesting Complex II under fluctuating light

Hamed Sattari Vayghan, **Paolo Longoni**

Institute of Biology, University of Neuchâtel

The Light Harvesting Complex II (LHCII) is constituted by a set of pigment-binding proteins embedded in the thylakoid membrane. These proteins contain a large part of the total chlorophyll of the plant. Dynamics of the LHCII are crucial for the adaptation of the plants to different environments. In fact, LHCII is functionally associated to both photosystem I and II. The fraction of the LHCII connected to each photosystem is dynamic, allowing a fine tuning of the electron transport chain. Furthermore, LHCII can act as a dissipator of the excess of light thereby protecting from photodamage. In *Arabidopsis thaliana*, the majority of the LHCII is composed by trimers of three isoforms: Lhcb1, Lhcb2 and Lhcb3. Both Lhcb1 and Lhcb2 can be phosphorylated, but only Lhcb2 phosphorylation appears to be essential to bind LHCII to photosystem I. The role of Lhcb1 in LHCII dynamics is less clear; however, its phosphorylation has an impact in vivo allowing a partial response also in absence of Lhcb2. We produced complete knock-out for Lhcb1 and Lhcb2, while the first results in a pale phenotype, the lack of Lhcb2 is compensated by Lhcb1. Furthermore, loss of Lhcb1 results in a de-phosphorylation of Lhcb2, while loss of Lhcb2 results in an over-phosphorylation of Lhcb1. The complete knock out plants for Lhcb1 and Lhcb2 were tested under fluctuating light. This revealed an increased susceptibility for the complete Lhcb1 knock out, visible as a growth delay, combined with a decrease in the photosynthetic efficiency and a loss of most of the major thylakoid

proteins. Loss of Lhcb2 did not result in any major defect in fluctuating light condition. Taken together, these results point at a very important role of Lhcb1 in protecting the plants during rapid light intensity changes.

LMNOPQRSTUVWXYZ

Engineering bacterial symbionts of nematodes improves biocontrol potential of the western corn rootworm

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⁴ Molekulare Biotechnologie, Buchmann Institute for Molecular Life Sciences (BMLS), Goethe-Universität Frankfurt, and LOEWE Translational Biodiversity Genomics (TBG)

⁵ Institute for Infectious Diseases, University of Bern

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Bacterial symbionts benefit their hosts by providing auxiliary biological functions. Despite their prevalence and importance across the tree of life, the potential to enhance their services through targeted biological engineering remains largely untapped. Here, we use an experimental evolution approach to generate *Photorhabdus* symbionts that resist plant toxins and thereby improve the capacity of their entomopathogenic nematode hosts to control an important agricultural herbivore pest. The efficacy of the nematodes to control the western corn rootworm, one of the most damaging maize pests on the planet, is reduced by benzoxazinoids, which are produced by plants and sequestered by the western corn rootworm for self-defense during feeding. We show that selecting *Photorhabdus* bacteria on benzoxazinoids results in genetic alterations that are associated with enhanced benzoxazinoid resistance. Selected bacteria are more efficient

at killing benzoxazinoid-containing western corn rootworm larvae. The evolution of benzoxazinoid resistance leads to a reduction of growth and symbiosis in some strains. However, we identified a *Photorhabdus* strain that is more resistant to benzoxazinoids and does not suffer from any reduction in growth or symbiosis. Increased benzoxazinoid resistance in this strain is shown to be caused by a mutation in the aquaporin-like channel *AqpZ*. Reestablishing symbiosis between this bacterial strain and its ancestral nematode host creates an enhanced entomopathogenic nematode strain that is more effective at killing benzoxazinoid-containing western corn rootworm larvae. Hence, this work provides an avenue for the improvement of biological control agents and demonstrates the power of forward evolution for the improvement of bacterial symbionts.

ABCDEFGHIJKLM

How to better predict long-term benefits and risks in weed biocontrol: An evolutionary perspective

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The fundamental area of uncertainty associated with biocontrol introductions is their potential evolutionary changes post-release. In 2013, we were confronted with the accidental introduction of the North American native ragweed leaf beetle *Ophraella communa* into Europe, which needed an urgent decision on how to respond to this unforeseen arrival of an oligophagous insect and potential biocontrol agent against common ragweed *Ambrosia artemisiifolia*, one of the most prominent plant invaders in Europe. Firstly, I will briefly summarize our recent findings from a multitude of ecological studies on the beetle's potential benefits and its potential risks for non-host plants in Europe. Secondly and in view of improving predictions for future long-term benefits and risks of this potential biological control program, we initiated a novel experimental evolutionary approach to assess the beetle's potential to select for resistant/tolerant ragweed populations, as well as the beetle's potential for evolutionary adaptation to novel biotic (sunflower) and abiotic (colder temperature for the yet unsuitable habitats in Central Europe, and considering climate change) conditions, using next generation sequencing, bioassay approaches and environment-phenotype correlations. I will present results of these studies from our demographic, phenotyping as well genomic analyses of both the experimental

ragweed (over 2 generations) and beetle (over 4 generations) populations, as well as of phenotyping *Ophraella* populations from the native (US) and introduced ranges (China and Europe).

This is the first attempt to rigorously and simultaneously assessing the evolvability of a biological control agent its target weed.

The role of AtABCG32 transporters in cutin formation

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The polyester cutin is a main constituent of the hydrophobic cuticle protecting aerial organs of land plants from biotic and abiotic stresses. Cutin formation requires the export of cutin precursors from the inside of cell to the apoplast where the cutin polyester is synthesized. Genetics point to a central role of ATP-binding cassette (ABC) transporters of the G family in this step. In *Arabidopsis*, the full-size transporter AtABCG32 as well as half-size transporters, such as AtABCG11, contribute to cutin formation. Genetics indicate redundant and non-redundant functions. However, their transport activities have not yet been elucidated.

For assessing the transport activities of ABCG transporters involved in cutin formation, several ABCG transporters of *Arabidopsis* and tomato have been expressed in *N. benthamiana* and their activity was assayed in protoplasts. ABCG32 as well as ABCG11 actively export mono 10,16 diOH C16:0-2-glycerol (2-MHG), but also free ω -hydroxy acids indicating that the esterification to glycerol

is not an essential substrate characteristic. Further investigations are under way. Since tomato fruits have a thick cuticle with 10,16 diOH C16:0 as main cutin constituent, the function of tomato ABCG32 homologous in cutin formation was studied *in planta*. Expression of the AtABCG32-homologue SlABCG42 in the *Arabidopsis pec1/abcg32* mutant complemented the petal cutin composition, including in the amount of 10,16 diOH C16:0. Fruit exocarps of *ABCG32_H-RNAi* plants knocked down in both *AtABCG32* homologues, *SlABCG36* and *SlABCG42*, had reduced amounts of 10,16 diOH C16:0, similar as the *Arabidopsis pec1/abcg32* mutant. Moreover, *ABCG32_H-RNAi* fruit cuticles had a reduction in the glycerol content in accordance with the export activity for mono 10,16 diOH C16:0-2-glycerol. The reduced amount of cutin leads to a thinner fruit cuticle and flatter epidermal cell shape as well as modifications of the biophysical properties of the cutins of *ABCG32_H-RNAi* fruits. In summary, the results demonstrate the conserved function of ABCG32 orthologs in the export of mono 10,16 diOH C16:0-2-glycerol and their essential role in the formation of a functional cuticle.

Allopolyploid radiation despite eco-genetic additivity of diploids

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Eco-genetic drivers of polyploid speciation and diversification remain elusive. To establish in face of their diploid progenitors, polyploid species are predicted to become genetically and ecologically divergent. Available studies have accordingly emphasized on the adaptive evolution of duplicated genes and the radiation of polyploids in novel habitats. Departure from the null hypothesis of diploid additivity into polyploids and the biological significance of observed differentiation have however rarely been tested. Using four diploid wild wheats that differentially combined into four allopolyploid species, we here show how historical, genetic and ecological constraints should be disentangled to appraise underpinnings of their radiation. Genetic variation at low copy genes supports genetic additivity, whereas comparative phylogeography and modelling of climatic niches indicate that allopolyploids are ecologically additive to their locally adapted diploid progenitors. Diploids further occupy only a small fraction of their potential distribution, while allopolyploids widely expanded to largely fill their suitable range. Such polyploid diversification despite conservative evolution appears mainly linked to selfish transposable elements that specifically deviate from genetic additivity and differentially accumulate among wild wheats, yielding conflicting interactions in hybrids and supporting reproductive isolation. Accordingly, endogenous drivers resulting from the additive merging of functional loci within an otherwise dynamic genome appear key to the success of allopolyploids under environmental changes.

PQRSTUVWXYZ

Lotus corniculatus hosts a surprisingly large diversity of nitrogen-fixing rhizobia in Switzerland

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Amongst the ca. 200 species that form the *Lotus* genus, *L. corniculatus* (birdsfoot trefoil) has the greatest distribution with specimens growing throughout most temperate regions of the world. In Europe, *L. corniculatus* is widespread and has colonized ecosystems exposed to notably different climate and soil constraints, ranging from subalpine meadows to city parks. Taking advantage of such quasi-ubiquitous geographic distribution, we selected *L. corniculatus* as a host to trap beneficial nitrogen-fixing rhizobia, which distribution and diversity in swiss soils are poorly documented. Accordingly, diversity of symbiotic rhizobia was assessed at altitudes and ecosystems as diverse as possible by collecting root nodules of *L. corniculatus* in 13 locations of Western and Southern Switzerland, across a Bellinzona to Geneva transect and between 200 to 2,000 m AMSL. Once isolated, nodule bacteria were characterized by mass spectrometry and DNA sequencing, and found to belong to a surprisingly large variety of *Mesorhizobium* species, several of which may represent novel lineages. When inoculated onto *L. corniculatus* cv. Oberhaunstädter seedlings that were grown under controlled conditions, representative isolates also displayed a range of phenotypic properties: From ineffective to fully proficient rhizobia. While core genomes of nodule isolates varied considerably, symbiotic genes appeared as more conserved, indicating transfer of symbiotic genes across strains with diverse genetic

backgrounds and selection of microsymbionts by host plants contributed to shape the pool of swiss microbes that associate with birdsfoot trefoil. Whether lower temperatures and shorter vegetation periods encountered by host plants in subalpine ecosystems select for specific rhizobia traits is one of the many questions we aim to explore.

ABCDEFGHIJKLMNNO P

From starch biosynthesis to chloroplast development – neofunctionalization of BRANCHING ENZYME1 (BE1)

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Branching enzymes introduce α -1,6-linked branches into starch, the plastidial storage carbohydrate of plants. Impairments in starch metabolism typically moderately affect plant growth. However, in case of BE1, one of the three branching enzymes in *Arabidopsis thaliana*, homozygous mutants arrest at the heart stage of embryogenesis, which has largely impeded the protein's characterization. Using immunoprecipitation, we recently identified three potential interaction partners of BE1, two of which have essential roles in plastidial RNA processing and translation. These data indicate a vital function in chloroplast biogenesis for BE1, rather than in starch metabolism. Consistent with this, embryo-specific expression of BE1 complements embryo lethality, but the rescued plants produce albino true leaves and are seedling lethal. Canonical starch-branching enzymes contain α -amylase domains and an appended carbohydrate-binding module (CBM). Although BE1 shares this domain structure, the catalytic site of its α -amylase domain is not conserved and previous attempts to detect glucan branching activity of BE1 failed. Nevertheless, BE1 versions with marked truncations of the α -amylase domains cannot complement the mutant phenotype. Likewise, BE1 variants carrying mutations in conserved tryptophan residues within the CBM or lacking the CBM altogether do not rescue embryo lethality. Together, our data suggest that BE1 may have

evolved from a starch-biosynthetic protein to a component of the plastidial gene expression machinery, where its CBM could act as a regulatory metabolite sensor or RNA binding platform. Potential CBM ligands and the functions of BE1-mediated protein-protein interactions are currently being investigated.

Nutrient exchange in arbuscular mycorrhiza: Carbon supply from host to fungus

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Development of arbuscular mycorrhiza (AM) requires fundamental reprogram-ming of root cells for symbiosis. The GRAS-type transcription factor in *Petunia hybrida*, ATA/RAM1, is required for gene induction in the host, and for morphogenesis of the fungal endosymbiont (1). To better understand the role of RAM1 in symbiosis, we have carried out a transcript profiling experiment by RNAseq of mycorrhizal plants vs. non-mycorrhizal controls in wild type and ram1 mutants (2). The results, and a comparison with similar data sets from various other AM associations including different host plants, show that fatty acid (FA) biosynthetic enzymes, lipid modifying enzymes are highly induced in mycorrhizal roots. In particular a glycerol-3-phosphate acyl transferase (GPAT) known as *REQUIRED FOR ARBUSCULAR MYCORRHIZA2* (RAM2) is highly induced. In addition, genes encoding a pair of half-size ABC transporters, *STUNTED ARBUSCULE* (STR) and STR2, is highly induced in mycorrhizal roots. RAM2, STR, and STR2 have been shown to be required for AM in *Medicago truncatula*, and surprisingly, closely related genes have been identified in the biosynthetic pathway of the extracellular lipid polyester cutin (3). The finding that AM fungi lack the fatty acid biosynthetic enzyme complex FAS1 suggested that AM fungi may receive lipids from their host (3), and indeed direct evidence for a transfer of lipids has been provided in *M. truncatula* and *Lotus japonicus*.

Here, we address carbon transfer from the host to the AM fungus by isotopic labelling with $^{13}\text{CO}_2$, and subsequent secondary ion mass spectroscopic analysis with subcellular resolution (nanoSIMS). We show that ram2 mutants accumulate large amounts of lipids in cells hosting fungal structures, consistent with a role of RAM2 in carbon delivery to the fungal partner through a lipidic intermediate.

- (1) Rich et al. (2015) Plant Physiology 168, 788-797.
- (2) Rich et al. (2017) BMC Genomics 18, 589.
- (3) Rich et al. (2017) Trends in Plant Science 22, 652-660.

Analysis of the LRX-RALF-FER signaling network involved in cell growth and cell wall integrity sensing

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Cell growth requires the coordination of cell enlargement with cell wall expansion and the deposition of newly synthesized cell wall material into the expanding cell wall. An elaborate system of sensors surveils cell wall integrity (CWI) and, if necessary, induces compensatory changes in cell wall structures and cell growth processes. Several of the *Catharanthus roseus* receptor like kinase1-like (*CrRLK1L*)-like receptor kinases such as THESEUS (THE) and FERONIA (FER) are involved in these activities. THE and FER are also receptors for RALF peptides, **R**APID **A**LKALINIZATION **F**ACTORS, that influence diverse processes such as Ca²⁺ dynamics and H⁺- pump (AHA) activity, which results in changes in the apoplastic pH that alter cell growth.

Leucine-rich repeat extensins (LRXs) are extracellular high-affinity binding sites of RALF peptides. LRXs of different vegetative tissues also function in a signaling pathway with FER in response to salt stress and to modify vacuole development that influences turgor-driven cell growth. As LRXs are tightly attached to the cell wall, the LRX-RALF-FER signaling module physically links the cell wall with the plasma membrane and influences CWI sensing.

LRX1 of *Arabidopsis* is expressed in root hairs and an *lrx1* mutant develops a root hair deformation phenotype. We have identified *rol16* (repressor of *lrx1_16*), a suppressor mutant of the *lrx1*-induced root hair defect that also suppresses the root

hair-less phenotype of *fer* mutants. A particular characteristic of *rol16* is the insensitivity to RALF1 but not to other RALF peptides, suggesting that *ROL16* function downstream of the LRX1-RALF1-FER signaling module. The detailed characterization of *rol16* will be presented.

Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth

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Plant reproduction relies on the highly regulated growth of pollen tubes for sperm delivery. This process is controlled by secreted RALF peptides, which are perceived by *CrRLK1Ls* receptor-like kinases and leucine-rich repeat (LRR) extensin proteins (LRXs). Here we demonstrate that RALF peptides are active as folded, disulfide bond-stabilized proteins, which can bind to the LRR domain of LRX proteins with nanomolar affinity. Crystal structures of LRX-RALF signaling complexes reveal LRXs as constitutive, redox-sensitive dimers. The LRR domain containing the RALF binding site is tightly linked to the extensin domain via a cysteine-rich tail. Our work reveals a complex signaling network by which RALF ligands may instruct different signaling proteins – here *CrRLK1Ls* and LRXs – through structurally different binding modes to orchestrate cell wall remodeling in rapidly growing pollen tubes.

Standing epigenetic variation speeds up plant adaptation

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There is some excitement in plant ecology and evolution that epigenetic variation could speed up adaptation to novel environments in the face of rapid global change. However, it is difficult to distinguish between rapid evolutionary change based on standing genetic vs. epigenetic variation. For example, using a novel reduced-representation bi-sulfite sequencing method, we found that multiple species showed rapid evolutionary changes in both methylation and DNA sequences in response to diversity-driven selection and concluded that all epigenetic differences could have been caused by genetic effects. We only know of one example where heritable adaptive changes in response to selection occurred without correlated genetic changes, i.e. within totally homozygous lines of *Arabidopsis thaliana*. However, in this case we could not find out how the epigenetic variation that selection could act upon arose in the first (or second) place. One possibility was that this epigenetic variation was reminiscent of an old hybridization event between different genotypes, whereby the gene(s) causing the epigenetic variation had been lost in the course of repeated selfing. Another possibility was that the

epigenetic variation arose during the course of the selection experiment. We therefore applied environmental stress to offspring of a single homozygous seed and applied artificial selection for small vs. large size over three generations but did not find any indication of selection response among the 150 tested populations. This indicated that even though rates of epimutations are much higher than genetic mutation rates, it still requires a population which has accumulated standing epigenetic variation for rapid adaptation to environmental change to become possible. In new experiments starting with single homozygous seeds we allowed replicated small vs. large populations to accumulate epigenetic variation over several generations and also formed genetically homozygous “epigenetic hybrids” between them. We will subject these populations to different selection pressures to test if large and hybrid populations show a stronger evolutionary response. For now, we can already report large phenotypic differences between plants from large vs. small populations and strong epigenetic “hybrid vigor”.

Machine learning of transcriptome data in natura: Drought as a trigger for flowering in aseasonal tropics

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Plants in naturally fluctuating environments, or in natura, are subjected to complex conditions. In order to understand and predict plant responses accurately in natura, we here focused tropical tree species by analyzing a large-scale time-course transcriptome data. Seasonal cues are widely used by plants for flowering, but are scarce in the aseasonal tropics of Southeast Asia. It has long been debated whether rainfall, temperature or solar radiation triggers flowering in tropical rainforests lacking seasonal cues. To address this question, we collected time-course genome-wide expression data from Macaranga trees for 20 months and quantified it using a de novo genome reference assembly. We analyzed the relation between climatic variables and expression levels of key flowering genes over time using “least absolute shrinkage and selecting operator” (LASSO) regression, a standard machine learning tool for variable selection. The combination of high rainfall followed by drought best predicted flowering. This supports the hypothesis that drought triggers flowering genes, and may be a vestige of past annual dry seasonality. The results of this analysis can be used to predict the flowering events under scenarios of global change, such as increased

frequency of droughts. Machine learning is a powerful tool to analyze temporal transcriptomic data that link environmental cues to plant phenotypic responses in natura.

Cryptic genetic variation with impact on crop productivity

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Genome editing technologies promise major advances in plant and animal breeding. However, a looming challenge of engineering desirable genetic variation across diverse genotypes is poor predictability of phenotypic outcomes due to unforeseen interactions with cryptic second-site mutations. In the model crop tomato, breeding with a mutation that causes loss of fruit abscission and improved harvestability frequently results in excessive flowering and reduced fertility due to interaction with a cryptic variant in a homologous gene. We found that a recently evolved tandem duplication carrying this cryptic second-site variant enabled breeders to neutralize negative epistasis on yield. By dissecting the mechanisms by which this structural variant restored normal flowering and fertility, we devised strategies that use CRISPR/Cas9 genome editing to predictably improve fruit harvestability. Our findings highlight the underappreciated impact of gene-by-gene interactions in targeted trait breeding, and underscore the need for a deeper characterization of cryptic genetic variation to unleash the full potential of genome editing in agriculture.

Rapid evolution of a plant invader in response to biological control and global warming

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Invasive alien plants together with their natural enemies from the native range used as biocontrol agents are ideal study system to address questions of whether and how fast organisms adapt to changing environments. Climate change is likely to impose further selection on invasive plant populations in interaction with the biocontrol process. In 2016, we started an experimental evolution study to get insights into the evolvability to a biocontrol insect and global warming of the European plant invader, *Ambrosia artemisiifolia*. In an ongoing field selection experiment in N-Italy, we grow artificial populations of *A. artemisiifolia* exposed to the recently introduced and potential biocontrol herbivore *Ophraella communa*, and a warming treatment (+3C°) in a two-by-two experimental design with five replicates. To test for evolutionary changes of this selection experiment, pooled samples from each of the 20 experimental populations are analysed over four years (a) for their genetic composition using next-generation sequencing (pool-seq) and (b) in various bio-assays. I will present the differentiated SNPs and their annotations from pool-seq analyses and metabolomic differentiations from an untargeted metabolomic analyses. I will also present some of our phenotyping results of the offspring plant performance and the quarantine preference and performance studies with *O. communa* on offspring plants from the field selection populations. These studies will improve forecasting of the biocontrol efficiency and spread of invasive alien plants in a changing world.

Genomics-based approaches to characterise and efficiently utilize genetic diversity for plant breeding

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Genetic diversity is the foundation of every successful plant breeding program. The recent advancements, not only in DNA sequencing and genotyping technologies but also in a variety of disciplines connected to plant breeding, allow to efficiently identify and utilize genetic variation for cultivar development. Using buckwheat (*Fagopyrum esculentum Moench*) and wheat (*Triticum aestivum L.*) as two contrasting examples, I will present how molecular breeding approaches can be used to precisely characterise plant genetic resources and increase the selection efficiency (and thus the genetic gain) in breeding. For buckwheat, a genotyping-by-sequencing approach was adapted to precisely describe allele frequencies at ~16,000 loci in pools of 100 individuals from 20 buckwheat accessions. These genome-wide allele frequency fingerprints were then used for detailed genetic characterisation of the 20 accessions and to conduct first steps towards genome-wide association studies. For wheat, the Illumina Infinium 25K SNP arrays was used to genotype a total of ~21 million genome-wide SNPs in 920 wheat lines. In combination, with phenotypic data of 20 commercially relevant traits measured for at least two years in four different environments, we used genomic selection to predict the performance of individual wheat lines at high accuracy.

The approaches presented here will contribute to mitigate the increasing challenges agricultural production systems will face in the coming decades.

Editorial (2019) Genomics and our future food security. *Nature Genetics* 51(2): 197-197.
Byrne, S., et al. (2013) Genome Wide Allele Frequency Fingerprints (GWAFs) of populations via genotyping by sequencing. *PLOS ONE* 8(3): e57438.

A pathogen effector protein promotes symplastic cell-to-cell trafficking by physical interaction with plasmodesmata-localized callose synthases

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Pathogen effectors act as disease promoting virulence factors by targeting specific host proteins with roles in plant immunity or cellular homeostasis. Here we show that the RxLR3 effector of the oomycete plant pathogen *Phytophthora brassicae* acts as a positive regulator of plasmodesmata (PD) connectivity. PD are cell-to-cell channels that play important roles in symplastic transport and communication. The aperture of PD is mainly regulated by deposition of the beta-1,3-glucan polymer callose catalyzed by callose synthases and its removal catalyzed by β -1,3-glucanases. We show that the RxLR3 effector localizes to PD and physically interacts with three closely related PD-associated callose synthases. In line with an inhibitory activity of RxLR3 the deposition of callose at PD is reduced in the presence of RxLR3 and cell-to-cell movement of free GFP is enhanced. The positive effect of RxLR3 on PD aperture is counteracted by the plant stress hormone salicylic acid that is known to trigger PD closure upon infection. *Arabidopsis* cells react to penetration by *P. brassicae* with enhanced plasmodesmal callose deposition that can be suppressed by constitutive expression of the RxLR3 effector. Our results demonstrate a virulence function of the RxLR3 effector as a positive regulator of PD transport and provide evidence for a competition between pathogen and host plant for control of cell-to-cell trafficking in plant-pathogen interactions.

An update on the role of *OCTOPUS-LIKE* genes in root development

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Plant roots are typical sink organs which are absolutely dependent on carbohydrate (sugar) import via the phloem. The long-distance transport of sugars occurs in the metaphloem sieve tubes. Close to the root tip, sugars are transferred to the protophloem sieve tubes, from which they get unloaded into the surrounding tissues.

Previously, we have identified the phloem specific *OCTOPUS (OPS)* gene as an important regulator of root protophloem differentiation in *Arabidopsis*. Mutations in *OPS* result in the stochastic appearance of undifferentiated cells in the root protophloem cell files and, most likely as a consequence of the phloem defects, reduced root growth. Our recent finding that *OPS* and *OPS-LIKE 2 (OPL2)* – a gene closely related to *OPS* – are redundantly important for proto- and also for metaphloem sieve tube development firmly establishes these genes as relevant regulators of phloem differentiation. Given the phloem defects, it is not surprising that root growth is even more impaired in *ops opl2* than in *ops*. However, since *OPL2* is expressed throughout the root meristem, at present we cannot rule out an additional direct effect of *OPL2* on root development. We have now started investigating the role of the three other *OPL* genes present in the *Arabidopsis* genome. Preliminary results point towards a more general role of these genes in root growth and will be presented.

UV-B photoreceptor signalling and responses

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Plants are able to perceive ultraviolet-B radiation (UV-B) using the UV-B photoreceptor UV RESISTANCE LOCUS 8 (UVR8) which activates a molecular signalling pathway leading to UV-B acclimation. The evolutionarily conserved UVR8 UV-B photoreceptor exists as a homodimer that monomerises upon UV-B absorption via specific intrinsic tryptophans. The UVR8 monomer interacts with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), an E3 ubiquitin ligase, initiating a molecular signalling pathway that leads to gene expression changes. This signalling output leads to UVR8-dependent responses including UV-B-induced photomorphogenesis and the accumulation of UV-B-absorbing metabolites that function as “sunscreens”. I will present our latest understanding of how UVR8 regulates UV-B acclimation and developmental decisions.

Regulation of plant immunity by phyto cytokines

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An important layer of plant immunity is achieved through the perception of pathogen-associated molecular patterns by cell-surface pattern recognition receptors (PRR), which leads to pattern-triggered immunity. It has recently emerged that endogenous plant peptides produced in response to damage or during infection can modulate pattern-triggered immunity, and are thus referred to as phyto cytokines. For example, we have recently shown that RALF peptides perception by the receptor kinase FERONIA regulate immune signaling by controlling the complex formation between the PRR FLS2 and its co-receptor BAK1 (Stegmann et al., Science 2017; Xiao, Stegmann, Han et al., Nature 2019). Here, we will report the identification of two novel families of phyto cytokines and our efforts to characterize their perception mechanisms. Together, this work illustrates the wide range of plant peptides that regulate environmental sensing.

Enhanced plant metabolic pathway annotation for natural product biosynthesis in UniProtKB/Swiss-Prot

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Swiss-Prot Group, SIB Swiss Institute of Bioinformatics, Geneva

Our group specializes in the development and maintenance of expert curated knowledge resources for the life sciences. These include the UniProt knowledgebase (UniProtKB, <https://www.uniprot.org>) a comprehensive, high-quality and freely accessible resource of protein sequences and functional information that covers thousands of plant species, and the Rhea knowledgebase (<https://www.rhea-db.org>), a resource of computationally tractable biochemical reactions.

Here we describe recent work designed to improve the utility of these knowledge resources for integrated computational and experimental analyses of plant metabolic systems, with a particular focus on natural product biochemistry. Plants produce various natural products with extremely diverse molecular structures and activities. These natural products may have interesting medicinal properties (as antibiotics, anti-cancer treatments, analgesics or immune-suppressive drugs), as well as applications in the agronomy (as insecticides, fungicides and more), food (as flavors or pigments for example) and energy sectors (as biofuels). Expert curation of natural product pathways in UniProt and Rhea provides a means to link these chemical structures to the underlying genomic sequence of the gene clusters that synthesize them, and presents new opportunities for genomic data mining for the discovery of new biosynthetic

routes for industry. We will present examples drawn from a wide range of natural product pathways and plants including the anti-malaria drug artemisinin in *Artemisia annua*, and morphine and derivatives analgesics in *Papaver somniferum* in UniProt using Rhea.

The relative importance of geography in structuring adaptive radiations in island-like systems: Contrasting *Dracula* (Orchidaceae) and *Aeonium* (Crassulaceae)

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Many plant clades have generated spectacular, large arrays of species in short amounts of time, but their major evolutionary drivers are poorly understood. In particular, the relative importance of geographic shifts (island-hopping) and convergent evolution in structuring such rapid radiations is poorly documented in macroevolutionary studies. A major reason is that phylogenetic studies have long been unsuccessful in reconstructing relationships among closely related taxa.

Here, we contrast two spectacular plant radiations in island-like systems: *Dracula* orchids of mountain ranges of South and Central America are restricted to “islands” of misty rainforests, and *Aeonium* (Crassulaceae), tree houseleeks occur on all islands of the Canaries (Macaronesia). Both are spectacularly species rich and morphologically highly diverse putative examples of adaptive radiations, where classic approaches to phylogeny reconstruction have largely failed. In both clades we ask: How frequent are biogeographic movements relative to the speciation rate? And how convergent are major aspects of vegetative and reproductive morphology?

Based on novel (*Dracula*) or recent (*Aeonium*) phylogenies using NGS techniques, we establish the rates of diversification, biogeographic movement, and trait evolution. Results indicate that *Aeonium*, with a generalist pollinator system, radiated primarily through island hopping and allopatric speciation, with little convergent evolution of gross morphology. In contrast, *Dracula*, with a highly specialized pollination system, speciated mostly within regions in a

pattern of geographically replicated convergent radiations. Jointly, these results suggest that the general importance of geography in structuring plant radiations may greatly differ among clades, depending on key aspects of their biology.

Changes in allelic frequencies of *Brassica rapa* under experimental evolution with selection by bumblebees

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The current pollinator decline profoundly modifies plant-pollinator interactions with significant impacts on floral variation and mating system. Recently, Gervasi and Schiestl (Nature Communication 2017) performed experimental evolution with fast cycling *Brassica rapa* plants during nine generations, and emphasized phenotypic evolution of floral traits driven by differences in pollinators. They showed that plants can adapt to bumblebees' preferences through floral trait evolution like size flower and fragrance within few generations. Based on this study, we track the genetic bases underlying these phenotypic evolutionary processes. The sequencing of parental replicates at the first and ninth generation identified 11.061 single nucleotide polymorphisms (SNPs) after filtering. We performed a genome-wide scan based on allelic frequencies changes along the genome of *B. rapa* revealing signature of selection for some loci. Our results suggest a rapid evolution in response to bumblebees' selection due to allelic frequencies changes out of standing genetic variation. While our study is the first step to understand the genetic bases of rapid plant adaptation to pollinator changes, it seems essential to deepen our knowledge on plant adaptation to pollinator communities in natural populations considering combined abiotic and biotic factors (as herbivores, co-flowering plant species, climate, soil).

Is Hsp70 recruited to re-potentiate heat-depolarized calcium channels in the plasma membrane of plants

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Particular cyclic nucleotide gated channels (CNGCs) in the plasma membrane of higher plants act as thermo-sensors. The land plant CNGC2 and CNGC4, which likely form hetero-tetramers, contain C-terminal cytosolic domains that can bind calmodulins and cyclic nucleotides. Upon temperature increase, the closed CNGC channels readily open to allow entry into the cytosol, of extracellular Ca^{2+} ions that can activate the bound calmodulin(s) and initiate a signaling cascade to produce heat-shock proteins (HSPs). Many HSPs act as molecular chaperones that protect proteins from heat-damages. Yet, within minutes, the heat-depolarized calcium channels become hermetically closed again, stopping the signaling for additional HSP production despite the ongoing heat-stress. Up to five hours back at low temperature are needed to fully re-potentiate the heat-depolarized CNGCs and to effectively produce a second fully potent heat-shock response.

Using the C-terminal cytosolic domain of CNGC2 as bait, we identified in a yeast-two-hybrid screen a unique plant DNAJC proteins. DNAJ co-chaperones generally target HSP70 onto protein complexes in need to be converted into structurally different complexes by the chaperone. Like Hsp60s, HSP70s can inject energy from ATP hydrolysis to convey, even under non-equilibrium conditions, stable misfolded proteins up a free energy landscape, and transiently convert them into a less stable native state. We thus hypothesized here that at non-HS temperatures this specific DNAJC is recruiting HSP70 onto the heat-depolarized relaxed closed

CNGCs to re-potentiate them into a different closed state with a higher free energy, poised to readily respond to a second heat-shock. We now investigate the mechanism by which these plant channels may become tensed again by the combined action of the DNAJC and HSP70. Having shot a first arrow, Wilhem Tell's muscles also needed to inject the energy of ATP hydrolysis to tense again his cross bow, and shoot his second arrow.

Feedbacks of plant identity and diversity on the diversity and community composition of rhizosphere microbiomes from a long-term biodiversity experiment

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Soil microbes are known to be key drivers of several essential ecosystem processes such as nutrient cycling, plant productivity and the maintenance of plant species diversity. However, how plant species diversity and identity affect soil microbial diversity and community composition in the rhizosphere is largely unknown. We tested whether, over the course of 11 years, distinct soil bacterial communities developed under plant monocultures and mixtures, and if over this time frame plants with a monoculture or mixture history changed in the bacterial communities they associated with. For eight species, we grew offspring of plants that had been grown for 11 years in the same field monocultures or mixtures (plant history in monoculture vs. mixture) in pots inoculated with microbes extracted from the field monoculture and mixture soils attached to the roots of the host plants (soil legacy). After 5 months of growth in the glasshouse, we collected rhizosphere soil from each plant and used 16S rRNA gene sequencing to determine the community composition and diversity of the bacterial communities. Bacterial community structure in the plant rhizosphere was primarily determined by soil legacy and by plant species identity, but not by plant history. In seven of the eight plant species the number of individual operational taxonomic units with increased abundance was larger when inoculated with microbes from mixture soil. We conclude

that plant species richness can affect below ground community composition and diversity, feeding back to the assemblage of rhizosphere bacterial communities in newly establishing plants via the legacy in soil.

Suberin dynamics during lateral root formation

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Plants roots take up essential nutrients and block out unwanted compounds from the soil by using a selective barrier in the roots known as the endodermis. Endodermis contains ring-shaped and lignin-based Casparian strips that act as diffusion barriers. Later in development, endodermal cells suberize to produce “patchy” suberization that eventually leads to a zone of continuous suberin deposition. The two impermeable polymers, lignin and suberin, affect paracellular and transcellular transport, respectively. Despite the chemically resistant nature of these polymers, the plant must locally remodel them during lateral root formation, as well as to seal the sites flanking lateral root primordia in order to prevent the infection by various pathogens. Using various genetic tools (Vermeer et al., 2014), we have identified several auxin-induced and differentiated endodermis-enriched candidate proteins which are important for removing the suberin during lateral root formation, as well as depositing it during endodermis maturation.

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