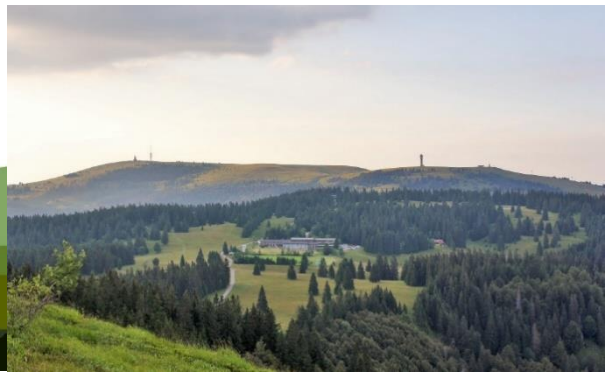
MAdLand Annual Meeting 2023

September 12th – 15th 2023
https://4science.de/madland

MAd Land
Molecular Adaptation to Land:
plant evolution to change

Abstract Book

Edited by Janine Fürst-Jansen, Jan de Vries, Stefan A. Rensing



Venue: Leistungszentrum Herzogenhorn
Black Forest Highlands at about 1,300m above sea level

Acknowledgements

MAdLand is funded by the DFG Priority Programme 2237. The meeting is organized by 4Science e.V. The scientific program is realized by the https://madland.science/ steering committee. Support by Eleva, Physiologia Plantarum, and BMG Labtech is gratefully acknowledged.

Thank you very much!

Contents

Programme committee

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Program

- All talks take place in the new seminar room (former swimming-pool).
- The Poster sessions take place in the gym.
- Breakfast, lunch, supper, coffee breaks and evening entertainment are located in the dining rooms/terrace.
- Best talk evaluations take place for ● PhD students and ● PostDocs.
- MadLand members ○

Tuesday, September 12th

13:00	Registration open
13:30	*Bus shuttle from Freiburg train station to venue (1 h transfer time)*
15:00	*Coffee and cake*
15:45 – 16:15	**Welcome remarks** (Stefan Rensing)
Session 1; Chair:	Jan de Vries
16:15 – 16:45	**Invited Talk 1: James Clark (Bristol)**, 20+10 min Genomic and morphological coevolution across land plants
16:45 – 17:15	**Invited Talk 2: Zoe Popper (Galway)**, 20+10 min Adapt-A-Wall: the role of cell walls in the transition to land
	Short talk presentations, 12+3 min
17:15	Talk 1: Lukas Pfeifer (Classen, Kiel) - *Evolution of arabinogalactan-proteins: Protein backbones seem to have evolved prior to characteristic glycosylation patterns*
17:30	Talk 2: Shreya Kalan (Buschmann, Mittweida) - *UNTANGLING The Evolution Process Of TANGLED1*
18:00	*Supper*
19:00 – 20:30	*Welcome reception* **/ poster session I (odd numbers)**

Wednesday, September 13th

7:45 – 9:00	*Breakfast*
Session 2; Chair:	Janine Fürst-Jansen
09:00 – 09:30	**Invited Talk 3: Grace Hoysted (Dublin)**, 20+10 min Mucoromycotina fine root endophyte fungi – phenology and function in vascular plants
	Short talk presentations, 12+3 min
09:30	Talk 3: Mung Hsia Foo (Nakagami, Cologne) - *Characterization of LysM protein in streptophyte algae*
09:45	Talk 4: Alexander Banguela-Castillo (Philippar, Saarbrücken) - *Evolution of plastid FAX (fatty acid export) proteins and the plants' conquest of land - molecular and metabolic adaptation*
10:00	Talk 5: Linus Wegner (Ehlers, Giessen) - *The evolution of plasmodesmata*
10:15	*Coffee break*
Session 3; Chair:	Tim Rieseberg
10:45	Talk 6: Christoph Michael Schwarze (Petersen, Marburg) - *Characterization of phenylalanine ammonia-lyase and 4-coumarate CoA-ligase in early-diverged plants*
11:00	Talk 7: Till Ischebeck (Ischebeck, Münster) - *Proteome plasticity during Physcomitrium patens spore germination – from desiccation tolerance to heterotrophic growth and reconstitution of photoautotrophy*
11:15	Talk 8: Katarina Kurtovic (Petrasek, Prague) - *CHARActerize: a study of Chara PIN auxin efflux carriers*
11:30	Talk 9: Vanessa Polet Carrillo Carrasco (Weijers, Wageningen) - *Insights into auxin responses gathered with Penium margaritaceum*
11:45	Talk 10: Katrin Philippar (Philippar, Saarbrücken) - *Structure and function of plastid FAX (fatty acid export) proteins*
12:00	*Lunch*
13:00 – 14:30	*Coffee break* / **Poster session II (even numbers)**

Session 4; Chair: Lukas Pfeifer

14:45 – 15:45 **Short talk presentations**, 12+3 min

14:45 Talk 11: Maike Hansen (Höcker, Cologne) - *Evolution of light signaling*
15:00 Talk 12: Tim P. Rieseberg (de Vries, Göttingen) - *CarotPhyte: Apocarotenogenesis is wired into oxidative stress mitigation networks conserved between streptophyte algae and land plants*
15:15 Talk 13: Stephanie Frohn (Schippers, Gatersleben) - *Involvement of CuZnSOD in evolutionary conserved mechanisms beneficial for plant terrestrialization*
15:30 Talk 14: Sigrun Reumann (Reumann, Hamburg) - Evolutionary dynamics of peroxisome functions and biogenesis enabling plant terrestrialization (PeroxEvo)

16:00 – 17:30 ***Hike to the top of Herzogenhorn, with group photo***

18:00 *Supper*

19:00 – 20:00 ***Optional slot for TAC meetings***

Evening *Get together*

Thursday, September 14th

7:45 – 9:00 *Breakfast*

09:00 – 12:00 **Oral presentations**

Session 5; Chair: Maike Hansen

09:00 - 09:30 **Invited Talk 4: Andreas Holzinger (Innsbruck)**, 20+10 min
Sexual reproduction in Zygnematophyceae - beneficial for terrestrialization?

09:30 – 10:15 **Short talk presentations**, 12+3 min

09:30 Talk 15: Nora Gutsche (Zachgo, Osnabrück) - *The bZIP transcription factor MpTGA has a dual role in sexual development and herbivore defense in M. polymorpha*
09:45 Talk 16: Carolin M. Heise (Hagemann/Schubert, Rostock) - *Interplay between carbon acquisition and osmo/ion regulation in C. braunii*
10:00 Talk 17: Peter Szovenyi (Szovenyi, Zürich) - *Pyrenoid formation and carbon concentrating mechanisms in hornworts: contrasting dynamics but parallel molecular underpinnings*

10:15 *Coffee break*

Session 6; Chair: Carolin Heise

10:45 Talk 18: Chiara Tessari (Theißen, Jena) - *MADS life on MAdLand: use of the CRISPR-Cas9 genome editing system in Marchantia polymorpha to delete exons crucial for MADS-domain protein tetramerization*
11:00 Talk 19: Clemens Rössner (Becker, Giessen) - *Evolution of transcriptional repressors (C1-1i zinc fingers) and their co-repressors in land plants*
11:15 Talk 20: Alisha Alisha (Szweykowska-Kulińska, Poznan) - *The role of MpSPL3 and MpSPL4 transcription factors in the development of liverwort Marchantia polymorpha*
11:30 Talk 21: Guillaume Brun (Wicke, Berlin) - *Transcriptional responses of bryophyte spore germination*

12:00 *Lunch*

13:00 – 14:30 *Coffee break* / **Poster session III (all posters)**

Session 7; Chair:	Erika Csisely
14:30 - 15:00	**Invited Talk 5: Martin Schattat (Halle/Saale)**, 20+10 min Plastid chains in Selaginella - A remnant of plastid division evolution?
15:00 - 15:30	**Cristo Gallardo / Deepti Varshney (Freiburg)**, 20+10 min Galaxy, Genome Zoo and NFDI4PLANTS: at your service
15:30	*Coffee break*
16:00 – 16:45	**MAdLand: past, present and future** Chairs: Jan de Vries and Stefan Rensing MAdLand timeline and changes for the second funding period
17:00	**Wrapup** Best talk prizes Final remarks
18:00	*Supper*
Evening	*Farewell event with Silent Disco*

Friday, September 15th

7:45 – 9:00	*Breakfast*
8:20	Shuttle from venue to Freiburg train station (1 h transfer time)

Invited Talk Abstracts

I1. Genomic and morphological coevolution across land plants

James Clark
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How can we infer the relationships among land plants? When did they first evolve? And how does this influence our understanding of trait evolution? Genome-scale data have supported a phylogeny where two major lineages of land plants exist as sister lineages: the vascular tracheophytes and non-vascular bryophytes. This changes our view of plant evolution and requires that we reconsider the way in which key traits and innovations are reconstructed. However, the fossil record of early land plants remains sparse and enigmatic, and so we can turn instead to comparative genomics to reveal the nature of the earliest land plants and their subsequent diversification. This is illustrated well by stomata – a key physiological innovation of land plants for which the evolutionary history is often obscured by uncertainty. I will discuss how palaeobiology, comparative genomics and phylogenetics can be integrated to infer the evolutionary history of stomata and plant morphological complexity as a whole.

I2. Adapt-A-Wall: the role of cell walls in the transition to life on land

Zoe Popper
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Most photosynthetic organisms share the feature that the majority of their cells are enveloped in polysaccharide-rich cell walls. Cell walls are important for plant and algal survival through their involvement in physiological processes including growth, development, and cell-cell communication, and defense. They are composed of polysaccharides, proteins (including structural proteins and enzymes), proteoglycans and phenolic compounds. Since cell wall composition alters wall properties, e.g. stiffness, the optimal composition may vary. As a consequence, cell walls are dynamic and are remodeled during cell division, expansive cell growth, and differentiation, as well as in response to environmental stresses e.g., desiccation and pathogen attack. The likelihood that cell wall composition was modified during the transition to land, where the ancestors of land plants were exposed to a new suite of selection pressures, is therefore intuitive. However, the freedom of individual wall components to evolve is an open question since they are necessarily interconnected and interdependent in order to maintain cell integrity, and to carry out their functions. Techniques, including immunolabelling, in situ and ex situ enzyme analyses, and biochemical and bioinformatic approaches, was used to explore the wall composition of non-vascular land plants and their closest extant ancestors and revealed that the transition to life on land was accompanied modifications in cell wall composition.

I3. Mucoromycotina fine root endophyte fungi – phenology and function in vascular plants

Grace A. Hoysted [1], Jeffrey G. Duckett [2], Martin I. Bidartondo [3,4], Silvia Pressel [2], Katie J. Field [5]
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Fungi have engaged in intimate symbioses with plants and have driven terrestrial biogeochemical processes since plant terrestrialisation >500 million years ago. Hitherto unknown nutritional mutualisms involving ancient lineages of fungi and nonvascular plants have been discovered, however their extent and functional significance in vascular plants remained uncertain. Using isotope tracing experiments, we show carbon-for-nutrient exchange between an early-diverging vascular plant (*Lycopodiella inundata*) and Mucoromycotina fine root endophyte (MFRE) fungi, providing evidence that MFRE play distinct functional roles at different plant life stages. We also demonstrate that the same fungal symbionts colonise neighbouring nonvascular and flowering plants, suggesting that these fungi have a much wider host range and ecological significance than previously thought. Most recently, using novel monoxenic cultures, we show the first unequivocal evidence that MFRE fungi are nutritional mutualists with a flowering plant in the absence of other micro-organisms. Our findings fundamentally change our understanding of the physiology, interrelationships, and ecology of underground plant-fungal symbioses in terrestrial ecosystems by revealing the nutritional role and plasticity of Mucoromycotina fungal symbionts in vascular plants.

I4. Sexual reproduction in Zygnematophyceae - beneficial for terrestrialization?

Andreas Holzinger [1], Charlotte Permann [1], Sebastian J. Antreich [1], Klaus Herburger [1,2], Pierre-Henri Jouneau [3], Clarisse Uwizeye [4], Denis Falconet [4], Eric Maréchal [4]
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Strategies of aquatic green algae to conquer land can be found in Zygnematophyceae, the sister clade of land plants. These green algae reproduce sexually by conjugation leading to the formation of resistant zygospores. They undergo a maturation process, where the male chloroplast is terminated. In both investigated species (Spirogyra *sp.* and *Zygnema vaginatum*), the zygospores are formed inside the female gametangia, but differ in the thickness of the mesospore. Serial block face-scanning electron microscopy (SBF-SEM) was applied on high pressure frozen/freeze substituted embedded samples of different Spirogyra sp., focussed ion beam SEM (FIB-SEM) on chemically fixed *Zygnema vaginatum* zygospores. Both techniques allowed a reconstruction of the zygospore formation at different maturation stages. In Spirogyra sp., chloroplasts were arranged as helices, and the male chloroplast was aborted and probably converted into vacuole-like compartments pre-vacuoles with a medium electron density. During zygospore ripening, Spirogyra sp. zygospores strongly upregulate lipid production and energy storage for later germination. In Zygnema, less lipid droplets are found and a massive, layered mesospore is formed. The visualization of 3D chloroplast architecture, the pyrenoid with inner thylakoids in gyroid arrangement, and the distribution of the large mitochondria, provide new insights in the dynamic reorganization processes during zygospore maturation. The unique cell wall architecture of both investigated genera is a structural innovation that permitted a shift to terrestrial habitats marked by frequent episodes of dryness. The acquisition of such structural feature represents a major evolutionary step towards land conquest by plants.

I5. Chloroplast Chains in Selaginella: A witness of plastid division evolution?

Martin Schattat, Daniela Rödel, Markus Jilge
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To thrive under diverse light conditions, land plants have evolved the remarkable ability to reposition their numerous independent chloroplasts within cells, ensuring optimal light capture while avoiding photodamage. Consequently, the development of precise mechanisms for plastid division and regulation holds significant implications for land plant evolution. Considerable progress has been made in understanding the protein components that govern division site initiation and thylakoid separation during plastid division. However, a crucial aspect that remains enigmatic is how the envelope membranes between nearly separated plastids are ultimately severed, leading to the finalization of division. Remarkably, the pioneering work of Haberland in 1888 sheds light on this intriguing topic. Observing chains of chlorophyll grains in the lycophyte *Selaginella kraussiana*, Haberland deduced that these chains resulted from incomplete plastid divisions, where the thylakoid systems are appropriately separated, while the isthmus remains intact. This intriguing observation positions *S. kraussiana* as a potential representative of an intermediate stage in plastid division evolution, offering a unique opportunity to study the elusive last step of plastid division. In this study, we demonstrate the establishment of transformation tools in *S. kraussiana* cells to visualize chlorophyll chains using fluorescence proteins. Through this approach, we validate Haberland's deductions made about these structures nearly 150 years ago, solidifying *S. kraussiana*'s potential as a model for studying plastid division evolution.

I6. Galaxy, Genome Zoo and NFDI4PLANTS: at your service

Cristobal Gallardo Alba, Deepti Varshney
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This talk aims to emphasize the usefulness of the Galaxy platform in facilitating bioinformatics analysis for plant biologists. The European galaxy server introduced here can be freely used.

Galaxy is an open-source, web-based platform for conducting data-intensive biomedical research. It allows to setup scientific workflows, data integration, data and analysis persistence, and comprises publishing tools, with the primary goal of enabling researchers who lack computer programming expertise to engage in computational biology. Galaxy offers various specialized tools and workflows tailored to different computational analyses. Moreover, the Galaxy platform promotes collaboration and knowledge sharing among scientists. Its features, including accessibility, reproducibility, and data-sharing capabilities, create a collaborative environment that encourages researchers to exchange data analysis protocols, reproduce results, and foster an open science culture within the community.

Examples
In particular in plant biology, isoform analysis holds significant importance due to alternative splicing that generates multiple isoforms from a single gene, significantly expanding the functional diversity of the plant proteome. Accurate identification and quantification of isoforms are fundamental for unraveling the complex regulatory networks governing plant development, stress responses, and specialized metabolism. Galaxy offers a diverse array of specialized tools and workflows tailored specifically for isoform analysis. These tools empower plant biologists to perform critical tasks such as transcript assembly, isoform quantification, and the identification of differentially expressed isoforms.

As another example, we will introduce Genome Zoo, also known as MAdLandDB, a comprehensive protein database accessible through the Galaxy platform. Focusing on non-seed plants and streptophyte algae, it delivers non-redundant, reliable genome sequences through BLAST and Diamond searches. The user-friendly Galaxy interface ensures effortless data access.

Finally, we will give a quick reminder to MAdLand members about leveraging the ARCs provided by NFDI4PLANT to store large amounts of annotated research data, as a means to make data and metadata available to and reusable by the community.

Short talk abstracts

MadLand members: **Tn.**

T1. Evolution of arabinogalactan-proteins: Protein backbones seem to have evolved prior to characteristic glycosylation patterns

Lukas Pfeifer, Birgit Classen
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Charophyte green algae (CGA) are assigned to be the closest relatives of land plants and can therefore help to enlighten crucial processes in colonization of terrestrial habitats. As arabinogalactan proteins (AGPs) are considered common for all land plant cell walls, we were interested when these special glycoproteins evolved in plant kingdom. With an analysis of available genomic and transcriptomic data from several plant species within the green lineage, we were able to show that AGP protein backbones seem to have evolved prior to characteristic AGP glycosylation. Carbohydrate attachment seem to have occurred firstly within the group of CGA. Our investigation therefore focussed on a number of algae from the Charales order, as well as on *Spirogyra pratensis* (Spirogyrales). AGPs were isolated via the use of β-Glc-Yariv reagent and their composition and fine-structure analysed by GC-FID/MS and AGP antibodies. Interestingly, no AGPs precipitated and no hydroxyproline was detected in all investigated members of the Charales. Within the Spirogyrales, the absence of arabinose and the presence of rhamnose side-chains (= RGP), together with occurrence of an AGP-like galactan backbone in Spirogyra Yariv-precipitates lead to the concept of a conserved galactan backbone structure with more flexibility in the decorating sugars.

T2. UNTANGLING The Evolution Process Of TANGLED1

Henrik Buschmann, Shreya Kalan
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During evolution from aquatic Streptophyte algae to land plants, cell division has experienced significant shifts. New structures like the cytokinetic phragmoplast and the preprophase band (PPB) of microtubules appeared while existing mechanisms like cleavage and the centrosomes disappeared. We aim to understand the molecular genetic alterations that underlie the adaptations of the cell division apparatus. Our analyses suggest that AIR9 and TANGLED1 genes play a role here. Their functions buffer each other in Arabidopsis, leading to dramatic cell division defects in the double mutants. While AIR9 is an ancient protein known from protists, TANGLED1 appeared much later in evolution. Here, we focus on TANGLED1 evolution, the first instances of the protein we find in bryophytes. We have no evidence for the gene gain through HGT. The predicted protein of bryophytes is large and already contains the conserved N-terminal helical domain. The C-terminal region of bryophyte TANGLED1 is partially disordered. Over the year, TANGLED1 protein contracts and becomes smaller. We hypothesize that the microtubule-binding domain of TANGLED1 evolved only at the base of the angiosperms. It consists of 2- 8 basic repeats, which appear to be Cyclin/CDK regulated. Our results suggest that in cell division genes, the ability for microtubule binding evolved repeatedly and independently, shaping the evolution of the cell division apparatus.

T3. Characterization of LysM protein in streptophyte algae

Mung Hsia Foo, Hirofumi Nakagami
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Basic Immune System of Plants, Max Planck Institute for Plant Breeding Research

Land plants (embryophytes) have evolved from streptophyte algae more than four hundred million years ago. Studies in angiosperms and bryophytes have revealed that cell-surface localized LysM (lysin motif) proteins serve as sensors for pathogenic and symbiotic microbes. The genome and transcriptome analyses revealed that streptophyte algae, the Charophyceae *Chara braunii* and the Zygnematophyceae *Spirogyra pratensis*, encode LysM kinase genes in their genomes. The function of LysM-kinase in streptophyte algae remain unknown. In this project, I aim to characterize the function of LysM-kinase in *Spirogyra pratensis* by taking biochemical, genetic, and multi-omic approaches. By exploring the molecular function of LysM protein in streptophyte algae, we will unravel how LysM protein evolved across the plant kingdom.

T4. Evolution of plastid FAX (fatty acid export) proteins and the plants' conquest of land - molecular and metabolic adaptation

Alexander Banguela-Castillo, Katrin Philippar
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Among the adjustments of plant metabolism to a terrestrial environment, changes in lipid homeostasis represent key steps. Fatty acid (FA) transport function of FAX proteins in the plastid envelope of Arabidopsis and Chlamydomonas has been shown to be crucial for cellular lipid homeostasis under normal and stress conditions such as cold acclimation. However, nothing is known about FAX proteins in those species, representing plant terrestrialization steps. Based on structural features, sequence motifs and a phylogenetic study (Banguela and Philippar, in preparation), we define the plant FAX-protein family in Viridiplantae. Besides their Tmemb14 membrane-spanning domain, the plastid-intrinsic FAX1-FAX3 contain distinct N-terminal stretches. Among them, the apolipoprotein-like α-helical bundle (apo) of FAX1/FAX2, which first appears in Charophyceae, is the most prominent. To evaluate FAX protein evolution in the light of metabolic adjustment to life on land, we thus aim to study molecular adaptation of plastid FAX proteins in bryophytes (*P. patens*, *M. polymorpha*) and possibly *M. endlicherianum*. With the emergence of the apo domain of plastid FAX1, most likely linked to promoter elements for auxin transcriptional regulation, we already identified molecular elements. Interestingly, the FAX apo domain seems to bridge the IE and OE membrane of chloroplasts for FA/lipid transport and might be involved in chloroplast-mitochondrium contacts for lipid exchange at phosphate starvation stress. Thus, we aim to identify membrane topology, formation of hetero-oligomeric complexes as well as in planta function of plastid apoFAX and FAX proteins. In the spotlight is the role in adaptation of lipid metabolism in response to stress.

T5. The evolution of plasmodesmata

Linus Wegner, Merlin L. Porth, Katrin Ehlers
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Intercellular exchange and communication via plasmodesmata (PD) likely was a prerequisite for the emergence of multicellular plants. In the well-studied seed plants, PD numbers at the cell interfaces are adjusted during organ development. Primary PD developing during cell division, are supplemented by secondary (sec.) PD formed postcytokinetically in existing cell walls. After identifying sec. PD formation via electron microscopy in the MAdLand model bryophytes we expanded our studies to ferns (*Ceratopteris richardii*) and lycophytes (*Selaginella moellendorffii*) supposedly unable to form sec. PD. We found sec. PD in Ceratopteris which supports an even earlier emergence of this mechanism in the MRCA of all land plants. Selaginella, however, has lost this ability, in contrast to the other lycophytes. In addition, we used confocal and electron microscopy to investigate the presence, morphology, and functionality of PD in species of the three ZCC grade classes (*Chara fragilis*, *Coleochaete scutata*, *Spirogyra pratensis*, *Zygnema circumcarinatum*). We showed the intercellular transport of ER-membrane localized DiOC6 in Chara and Coleochaete, suggesting the presence of a desmotubule-like structure to connect the ER of adjacent cells - similar to land plant PD. This morphological similarity points at a homologous rather than an analogous origin of PD in streptophyte algae and embryophytes. Our results support the loss of PD with the unicellular ancestor of the Zygnematophyceae.

T6. Characterization of phenylalanine ammonia-lyase and 4-coumarate CoA-ligase in early-diverged plants

Christoph Schwarze, Maike Petersen
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With the evolution of early-diverged plants making the transition from water to land it became increasingly important for the plants to gain resilience against environmental influences such as UV-light, predators and water loss. For this purpose, secondary metabolites derived from the phenylpropanoid pathway emerged in order to help the plants to cope with these conditions. Two of the enzymes responsible for the formation of these metabolites were examined with regard to their substrate specificities and enzyme kinetics: phenylalanine ammonia-lyase (PAL) catalyzing the reaction from L-phenylalanine to *t*-cinnamic acid via elimination of ammonia and 4-coumarate CoA-ligase (4CL) catalyzing the activation of 4-coumaric acid to 4-coumaroyl-CoA. The investigated organisms harboring these enzymes were the green alga *Chara braunii*, the bryophyte *Physcomitrium patens* and the liverwort *Marchantia polymorpha*. Photometrical characterization following expression of recombinant proteins of PAL showed acceptance of both L- and D-phenylalanine as well as L-tyrosine and L-histidine for several isoforms, L-phenylalanine being the best accepted substrate in every case. Characterization of 4CL showed for most isoforms a distinct acceptance of caffeic, 4-coumaric, cinnamic and ferulic acids and for some also a slight acceptance of sinapic and 4-hydroxybenzoic acids.

T7. Proteome plasticity during *Physcomitrium patens* spore germination – from desiccation tolerance to heterotrophic growth and reconstitution of photoautotrophy

Lea Hembach, Philipp W. Niemeyer, Kerstin Schmitt, Jaccoline M. S. Zegers, Dennis Brandt, Janis J. Dabisch, Oliver Valerius, Gerhard H. Braus, Markus Schwarzländer, Jan de Vries, Stefan A. Rensing, Till Ischebeck
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The establishment of seeds is considered a milestone in plant evolution. A major proportion of the protein networks underpinning desiccation tolerance, the accumulation of storage compounds, and the regulation of dormancy have, however, likely evolved much earlier. The same is the case for the molecular program that drives the transition from heterotrophic offspring to the autotrophic plant. We hypothesized that a comparison with a bryophyte representing a distinct evolutionary lineage to seed plants may reveal the origin of components of the "seed program". Therefore, we investigated the proteome of five timepoints of moss (*P. patens*) spore germination as well as protonemata and gametophores, and compared it to previously published Arabidopsis proteome data during seedling establishment. This quantitative comparison showed that not only spores are functionally related to seeds but also the functional similarity of germinating spores and young seedlings. We observed remarkable similarities with regard to desiccation tolerance, lipid droplet proteome composition, control of dormancy, and the metabolic pathways that transform fatty acids into sugars. However, there were also striking differences. For example, the spores of *P. patens* did not harbor any obvious storage proteins.

T8. CHARActerize: a study of Chara PIN auxin efflux carriers

Katarina Kurtovic [1], Stanislav Vosolsobe [1], Daniel Nedved [2], Pavel Jelinek [1], Jan Petrasek [1,2]
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The directional transport of auxin, mediated by PIN efflux carries has been extensively studied in land plants. However, their role and function in streptophyte algae remain poorly understood. The genome of complex alga *Chara braunii* encodes for six PIN homologs, the highest number among all streptophyte algae. Here we present our results on the characterization of three auxin efflux carriers in this multicellular alga. We assigned them names CbPINa, b, and c. A heterologous expression in tobacco BY-2 cells was used to study their intracellular localization, complemented with radioactively labeled auxin transport assays. GFP-tagged Chara PINs show localization both on the endoplasmic reticulum and plasma membrane, with the activity in the transport of auxin. Furthermore, we performed an immunolocalization study of CbPINa and CbPINc with homologous antibodies in the internodal cells of *Chara braunii*. Finally, we show that auxin promotes the cytoplasmic streaming velocity of germinating oospores in an ultrafast manner. Overall, we demonstrate that Chara PINs are able to transport auxin, that they are localized to the plasma membrane and that Chara reacts to externally applied auxin. These findings together provide insight into a conserved function of PIN auxin efflux carriers between land plants and streptophyte algae. The research was supported by the Charles University Grant Agency (nr. 289523).

T9. Insights into auxin responses gathered with *Penium margaritaceum*

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A largely unexplored question in biology is how hormone response networks emerged during evolution. Here, we target this question by studying the origins of the auxin signaling pathway from plants. We focus on revealing the molecular and cellular mechanisms in response to auxin in streptophyte algae, an extant group of green algae sister to the land plants. Some of the ancestral components of the plant auxin response system are present in streptophyte algal members, suggesting that the system was already present in a last common ancestor. However, most current knowledge about the origin of auxin responses is based on genomic and transcriptomic information. To shed light on this matter, we use the Zygnematophycea *Penium margaritaceum* as a model system with a combination of biochemical, transcriptomic, and microfluidics-based phenotyping approaches.

T10. Structure and function of plastid FAX (fatty acid export) proteins

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In plants, fatty acid (FA) synthesis occurs in the plastid stroma and thus requires subsequent FA export for lipid assembly in the endoplasmic reticulum (ER). In this context, we described the membrane-intrinsic protein FAX1 to mediate FA-export across the plastid inner envelope (IE). In Arabidopsis, FAX1 function is crucial for pollen cell wall formation, pollen tube growth and thus male fertility. Further, At-FAX1 affects plant biomass and cellular lipid homeostasis, including ER-based triacylglycerol (TAG), phospholipid and ketone wax assembly (Li et al., 2015). In addition, our most recent data show that At-FAX1 is a key player in cold acclimation (unpublished) as well as together with At-FAX3 in seed/embryo development and rosette leaf growth (Bugaeva et al., 2023). Since in comparison to fax1 single knockouts, fax1/fax3 double mutants are seedling lethal and not able to develop mature rosette leaves, we conclude that in seed plants FAX1 and FAX3 function together in vegetative leaf growth, most likely by formation of FAX1/FAX3 hetero oligomeric complexes. The latter is supported by membrane topology and protein/protein interaction analysis. Besides FAX1-FAX4 in plastids of seed plants, we find FAX5/6 and FAX7 to be targeted to ER/secretory pathway membranes. In Chlamydomonas, we can show that also in green microalgae the function of FAX1 and FAX5/6 is crucial for TAG oil production (Peter *et al*., 2022). Further, all Arabidopsis plastid FAX and FAX5/6 proteins can complement for FA-transport function in yeast. Overall, two basic sets of FAX proteins in plastids and secretory pathway membranes with corresponding structure/function appear to be conserved in Chlamydomonas and Arabidopsis: namely FAX1 + FAX5/6 and FAX3 + FAX7.

T11. Evolution of light signaling

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During terrestrialization, plants were exposed to severely altered light conditions, which required major molecular adaptations in order to ensure survival. Therefore, studying light signaling mechanisms in bryophytes and charophyte algae might enable the identification of light signaling components that were crucial for the conquest of land. Thus, we use the moss *Physcomitrium patens* and the charophyte algae *Mesotaenium endlicherianum* as model organisms to study the evolution of light signaling. We mainly focus on the COP1/SPA complex, which is a well-studied repressor of light signaling in Arabidopsis. There it acts as an E3 ubiquitin ligase to mark positive regulators of light signaling for degradation in the 26S proteasome. In order to study the evolutionary conservation of the COP1/SPA complex we performed interaction studies with Physcomitrium and Mesotaenium homologs of COP1 and SPA as well as other known light signaling proteins. Additionally, Ppcop1 and Ppspa mutants were generated and used for phenotypic analyses. These revealed that the Physcomitrium mutants are affected in many traits such as gametophore development, phototropism, gravitropism and chloroplast development. Additional analyses on the chloroplast phenotype hint to GLK transcription factors as putative targets of the COP1/SPA complex in Physcomitrium. Furthermore, we aim to unravel the mechanisms regulating GLK function through the COP1/SPA complex in Physcomitrium and Mesotaenium.

T12. CarotPhyte: Apocarotenogenesis is wired into oxidative stress mitigation networks conserved between streptophyte algae and land plants

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In an ever-changing environment, we need to understand the most transformative processes of Earth's history. One of these events occurred about 500 MYA – plant terrestrialization. We used a multi-dimensional approach to assess conserved environmental stress hubs in the closest algal relatives the Zygnematophyceae and land plants. We subjected *Zygnema circumcarinatum*, *Mesotaenium endlicherianum*, and the moss *Physcomitrium patens* to heat, cold and highlight stress and investigated their metabolomes (chlorophylls, chlorophyllides, carotenoids (RP-C30-HPLC-UV-Vis) and apocarotenoids (HS-SPME-GC-MS)), global differential gene expression dynamics, photophysiology and morphology in time-series experiments. Our findings enable a comparative framework for oxidative stress mitigation hubs across more than 600 million years of streptophyte evolution. We shed light on the role of (apo)carotenogenesis in plant terrestrialization. The wiring of this framework is further assessed by a combination of carotenoid synthesis inhibitor treatments, which yielded distinct growth effects and heterologous enzyme characterization. Applying a bouquet of methods our data pinpoint conservation and relevance of several stress-mitigation mechanisms that aided the singularity of plant terrestrialization.

T13. Involvement of CuZnSOD in evolutionary conserved mechanisms beneficial for plant terrestrialization

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As life evolved in the presence of reactive oxygen species (ROS) and was further challenged by two consecutive great oxidation events, it is not that remarkable that ROS are deeply intertwined into the physological, morphological and transcriptional responses of organisms. Copper zinc superoxide dismutases (CuZnSODs) evolved around the first great oxidation event and have next to their classical role in ROS detoxification also important roles in signaling and transcriptional regulation. Here we addressed the role of cytosolic CuZnSODs in early land plant evolution. Inhibition of CuZnSOD in *Marchantia polymorpha*, *Physcomitrium patens* and *Arabidopsis thaliana* causes a reduced development and growth rate indicating CuZnSOD is important for these processes. RNA-Seq and an untargeted metabolome analysis unraveled besides a general ROS-homeostasis perturbation, developmental specific targets being reactive to the treatment, highlighting an evolutional conservation of the moonlighting function of cytosolic CuZnSOD. CRISPR mutation lines in Marchantia which does only have one isoform of CuZnSOD, seem to be impaired in their sexual reproduction strengthen the hypothesis that the evolvement of cytosolic CuZnSOD has been a supportive factor for the development of embryophyta during evolution.

T14. Evolutionary dynamics of peroxisome functions and biogenesis enabling plant terrestrialization (PeroxEvo)

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Despite considerable progress, we are only in the beginning of understanding the cell biological dynamics in streptophyte algae that paved the way for land colonization. Peroxisomes are important cell organelles not only for fatty acid β-oxidation, photorespiration, and ROS homeostasis, but also for hormone biosynthesis, and abiotic stress tolerance. In this research project, we want to identify key innovations of peroxisomes that were required for plant terrestrialization. We first addressed (i) to what extent peroxisome functions known from land plants are conserved in streptophyte algae and (ii) in which order they evolved. Using the protein landscape of Arabidopsis peroxisomes, we analyzed the conservation of matrix proteins and peroxisome localization at genome scale in streptophytes in comparison to chlorophytes. Accordingly, several peroxisome functions of land plants are conserved in chlorophytes. More advanced peroxisome functions, however, apparently evolved either after the divergence of Mesostigmatophyceae (e.g., fully established photorespiration), after that of Klebsormidiophyceae (e.g., NADPH production), or after the branching of Charophyceae (e.g., benzaldehyde biosynthesis). Furthermore, our data newly indicate that the peculiar, peroxisome-specific mechanism of "piggy-back import" may have been crucial to allow the simultaneous re-direction of neighboring pathway enzymes into peroxisomes. In the new research project, we will use all available genomes and transcriptome data of streptophyte algae for conservation analyses of peroxisomal matrix proteins from land plants. To uncover lineage-specific novel functions, we will predict the entire matrix proteome of peroxisomes for 10 selected genomes of streptophytes in comparison to chlorophytes (WP 1). Peroxisome targeting of proteins from selected streptophytes will be verified experimentally, focusing on (i) predicted peroxisomal proteins with non-canonical peroxisome targeting signals type 1 and type 2 (PTS1 and PTS2) and (ii) proteins of specific metabolic pathways and from extant species that are located close to the predicted point of divergence in subcellular compartmentalization. The genes will be cloned from streptophytic model algae and expressed as fluorescent fusions first in Arabidopsis. To allow comprehensive analyses of subcellular targeting in Zygnematophyceae, we will create a set of transient expression vectors for the MadLand2 community to advance the establishment of *Spirogyra pratensis* as a new model alga (WP 2). We will furthermore address whether the peculiar, peroxisome-specific mechanism of "piggy-back import" may have been crucial to allow the simultaneous re-direction of neighboring pathway enzymes into peroxisomes (WP 3).

T15. The bZIP transcription factor MpTGA has a dual role in sexual development and herbivore defense in *M. polymorpha*

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Terrestrialization necessitated the adaption to various challenges leading to the development of physiological and morphological novelties in land plants mediated by changes in gene regulatory networks. TGA transcription factors (TGA), regulators of developmental and stress-related processes in seed plants, already exist in streptophyte algae. Angiosperm TGA activities are often modulated via interaction with land-plant specific NPR-like co-factors, which mainly act together in stress responses. In early diverging land plants, TGA functions and the impact of NPR-like proteins on TGA activities are unknown. Therefore, we investigated the function of the sole TGA, MpTGA, and its potential co-factor MpNPR in the liverwort *M. polymorpha*. Surprisingly, mutant analyses revealed a crucial MpTGA role in sexual organ formation together with MpNPR exhibiting a novel MpTGA co-activator function in this developmental process. Independently of MpNPR, MpTGA regulates the formation of oil bodies, a synaptomorphic feature of liverworts rich in secondary metabolites with protective activities against herbivores demonstrating a dual MpTGA function in sexual reproduction and herbivore defense. We will discuss the impact of co-factors in the diversification of TGA TF functions.

T16. Interplay between carbon acquisition and osmo/ion regulation in *C. braunii*

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During the last years it has been shown that charophytes can grow in a wide range of environments such as ultraoligotrophic to hypertrophic waters of different salinity, acidity, and alkalinity. Most photosynthetic organisms in these environments are challenged by the inorganic carbon (Ci) delivery, i.e., limited CO_2-availability and elevated presence of bicarbonate (HCO_3^-) in freshwater. For Chara, the passive uptake of negatively charged HCO_3^- will be insufficient due to the very negative membrane potential of these cells. To focus on the interplay between Ci acquisition and osmo/ion regulation in the model *C. braunii*, we exposed the algae to elevated levels of $NaHCO_3^-$ and analyzed its photosynthetic performance. In short-time periods, HCO_3^- can be efficiently used for photosynthesis shown by an increased maximal electron transport rate, however, this rate decreases in just 24 h. Algae cultivated in ambient air and with enhanced Ci supply (HCO_3^- addition, aeration with CO_2-enriched air) showed diminished trends in growth for HCO_3^-, consistent with its restricted use for photosynthesis. These results suggest that dissolved CO_2 could be the preferred Ci species for photosynthesis. We also analyzed the stable C isotope composition (δ13C) of *C. braunii* at different Ci conditions, which was suggestive of CCM activity because of the elevated δ13C values under ambient Ci conditions. In contrast, the δ13C values in algae under CO_2 or HCO_3^- supplementation resemble that of C3 plants, confirming the absence of CCM activity under such conditions. Recent work has focused on investigating the physiological and transcriptional response to different Ci conditions as well as the role of transport mechanisms in response to salt stress in C*. braunii*.

T17. Pyrenoid formation and carbon concentrating mechanisms in hornworts: contrasting dynamics but parallel molecular underpinnings

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Biophysical carbon concentrating mechanisms (CCMs) operating at the single-cell level have evolved independently in various lineages of eukaryotic algae and a single land plant lineage, the hornworts. An essential component for an efficient eukaryotic CCM is a pyrenoid, a specialized compartment inside the chloroplast that mainly comprises the CO2-fixing enzyme RuBisCO. Information on pyrenoid biology and CCM is primarily available for the unicellular green alga, *Chlamydomonas reinhardtii*, suggesting that both pyrenoid formation and CCM is highly dynamic and inducible by low CO2 concentrations. In contrast to *C. reinhardtii*, molecular underpinnings, inducibility and dynamics of the hornwort CCM and the pyrenoids are poorly understood. To start investigating molecular underpinnings of the CCM in hornworts we used a combination of methods including (1) protein co-IP of pyrenoid components, (2) localization of candidates homologous to CCM genes in Chlamydomonas, and (3) CO2 assimilation measurements in pyrenoid-bearing and pyrenoid-free species. We provide evidence that the scaffolding candidate and the RuBisCO co-localize using fluorescent reporter lines but pyrenoids are less dynamic than in *C. reinhardtii*. We further found that the carbon anhydrase homolog (CAH3) is localized to the pyrenoid, while the LCIB hornwort homolog is less intimately linked to the pyrenoid than in Chlamydomonas. Surprisingly, we observed that pyrenoid formation and subcellular localization of CAH3 and LCIB do not react to changing CO2 concentrations, darkness or H2O2 as it is observed in *C. reinhardtii*. Our results imply that the pyrenoid-based CCM of hornworts is characterized by a mixture of Chlamydomonas-like as well as hornwort-specific features which is line with their independent evolutionary origin. Furthermore, our study suggests that hornwort CCM may be less dynamic than that of *C. reinhardtii*.

T18. MADS life on MAdLand: use of the CRISPR-Cas9 genome editing system in *Marchantia polymorpha* to delete exons crucial for MADS-domain protein tetramerization

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MADS-domain transcription factors (MTFs) are important developmental control proteins that underwent major evolutionary changes during the transitions of plants to land. In the stem group of extant land plants, duplication of an ancestral MIKC-type gene occurred and led to the evolution of two lineages: MIKC*-type and MIKCC-type MTFs (M*-MTFs and MC-MTFs, respectively). In the gene lineage encoding MC-MTFs a duplication of the last K-domain encoding exon generated a strong tetramerization interface. By using the liverwort *Marchantia polymorpha* as a model system we are about to clarify the functional implication of tetramer formation and its role during plant terrestrialization. *M. polymorpha* is a well-suited model system for our analysis as there is only one MC-MTF encoding gene (MpMADS2) in its genome. We have previously shown that the sections of MC-MTFs encoded by the last two exons of the K-domain (exons 5 and 6) play a crucial role in tetramerization. Using CRISPR-Cas9 genome editing system we successfully generated multiple isogenic lines of mutated plants missing either exon 5, exon 6 or both exons. In vitro experiments showed that the deletion of exon 5 impedes MpMADS2 tetramerization. Therefore, the phenotype of plants missing exon 5 will now be compared through the whole life cycle of M. polymorpha to the phenotypes of plants missing exon 6 or both exons, and to that of mpmads2 knockout plants and wild-type plants. These analyses will reveal which functions of the MpMADS2 protein depend on tetramerization and which don't.

T19. Evolution of transcriptional repressors (C1-1i zinc fingers) and their co-repressors in land plants

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In angiosperms, C1-1i zinc finger (ZF) transcription factors are, next to other functions, involved in the development of floral organs. The Arabidopsis proteins JAGGED, KNUCKLES and ZFP8 are e.g. crucial for boundary formation between floral organs and floral meristem termination. They can interact with TOPLESS-like (TPL) co-repressors, eventually leading to the epigenetic silencing of target gene expression. C1-1i ZFs are broadly conserved in land plants but their functions in non-flowering plants are largely unknown. However, it has been shown recently that TPL orthologs in *Physcomitrium patens* are necessary for the transition from two- to three-dimensional growth, a key feature for reproduction on land. We assume that C1-1i ZFs and TPL-like co-repressors are involved in the evolution of the development of structures dedicated to sexual reproduction. As first step to explore this possibility, we inferred phylogenetic trees including major land plant lineages of C1-1i ZFs and TPL-like genes and compared their expression pattern. Even though reproductive structures are often not directly comparable between different plant lineages, our findings indicate a possible early recruitment of certain C1-1i ZFs in regulatory networks guiding sexual reproduction. As a next step, we plan functional analyses in non-seed plants including Ceratopteris richardii and Physcomitrium that might lead to a better understanding of how land plant sexual reproduction has evolved.

T20. The role of MpSPL3 and MpSPL4 transcription factors in the development of liverwort *Marchantia polymorpha*

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The SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) family of transcription factors is functionally diverse in controlling a number of fundamental aspects of plant growth and development. Each SPL gene encodes protein with the highly conserved 76-78 amino acids DNA binding domain, known as SBP domain. In angiosperms, *Arabidopsis thaliana* and Oryza sativa, almost all SPL family members have been functionally characterized. While amongst bryophytes, only three out of 13 and two out of four SPL family members have been functionally characterized in moss, *Physcomitrium patens* and liverwort, *Marchantia polymorpha*, respectively. Since the SPL genes exist amongst plants only, hence, it is imperative to understand their functions amongst basal lineages of land plants to gain understanding of their evolution. *M. polymorpha* has emerged as a model plant to study complex biological processes including physiological, developmental and stress-induced cellular responses. As object of our study, we have chosen *M. polymorpha* MpSPL3 and MpSPL4 genes which yet were not characterized functionally. In order to understand their roles, first we investigated MpSPL3 and MpSPL4 expression profile by combining GUS reporter approach together with RT-qPCR analysis. Both techniques revealed ubiquitous expression of these two genes during the vegetative as well as reproductive phase of Marchantia life cycle. Further, to gain information on MpSPL3 and MpSPL4 genes function, knockout mutant plants were generated via CRISPR/Cas9 and used for phenotypic analyses. The obtained knockout plants showed strong growth retardation as compared to wild type plants. Therefore, we designed artificial miRNA to knockdown the expression levels of MpSPL3 and MpSPL4 genes. To study how the MpSPL3 and MpSPL4 protein excess will influence the plant development, we have also prepared overexpression lines of both genes. Overall, our studies on Marchantia SPL genes will allow insights into the primary functions of SPL transcription factors in the representative of liverworts.

T21. Transcriptional responses of bryophyte spore germination

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Plants ensure that germination of their spores or seeds occurs at the right time and place. Many studies suggest that environmental regulation of seed and spore germination involves identical major factors, including temperature ranges and phytochrome-mediated photoreversible responses. However, little is known about the genetic programs involved in spore germination in non-vascular plant lineages. The overarching goal of this project is to assess the progression of gene regulatory networks for spore germination in non-vascular land plants. As a first keystone, we analyzed transcriptional responses of spore germination on the basis of time-course RNA sequencing data using the three model bryophyte species *Marchantia polymorpha* (liverwort), *Physcomitrium patens* (moss), and *Anthoceros agrestis* (hornwort). Time increments, distributed evenly over germination under fixed environmental conditions, were adjusted so that differences in germination time between *M. polymorpha* (48 h) and the other two species (144 h) were normalized. A regression-based approach was used to cluster significant gene expression profile differences. This presentation will position the major transcriptional events, whether common or distinct in temporality or functionality, taking place during bryophyte spore germination.

Poster abstracts

MadLand members: **Pn.**

P1. Fern cell walls: Structural investigations and the evolution of arabinogalactan-proteins

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Recent euphyllophytes belong to two clades, the ferns and the seed plants. The ferns comprise about 12,000 extant species and evolved about 430 million years ago. The composition of fern cell walls is still not fully understood and especially knowledge on fern arabinogalactan-proteins (AGPs) is strongly limited. Therefore, we isolated and characterized different cell wall fractions and especially AGPs from the leptosporangiate ferns *Azolla filiculoides*, *Salvinia molesta* and *Ceratopteris richardi*i. The carbohydrate moiety of seed plant AGPs consists of a galactan backbone including mainly 1,3- and 1,3,6-linked pyranosidic galactose (Galp), which was also confirmed for the investigated fern AGPs. In contrast to seed plant AGPs, an unusual methylated sugar (3-O-methylrhamnose) was part of fern AGPs. Besides terminal furanosidic Ara (Araf), the main linkage type of Araf in the three ferns was 1,2-linked Araf, whereas in seed plant AGPs 1,5-linked Araf is often dominating. Comparison of AGP linkage types across the streptophyte lineage by PCA analysis revealed groupwise clustering of AGPs in relation to the evolutionary positions by special AGP features. Bioinformatic search for AGP glycosyltransferases and AGP protein backbones was performed to complement our analytical results. Knowledge on cell wall diversity contributes to our understanding of plant evolution. Due to the crucial phylogenetic position as sister to land plants, ferns are key for understanding cell wall evolution.

P2. Terrrestrial filamentous Zygnematophyceae: Occurrences and effect of culture conditions on growth and morphology

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Filamentous Zygnematophyceae are often found in freshwater habitats. What is perhaps not so well known is that, like other Zygnematophyceae, they also occur terrestrially. We have been specifically looking for terrestrial Zygnematophyceae in recent years. In this contribution we document the occurrence of terrestrial Mougeotia and Zygnema species in the Eifel and the growth of different Zygnematophyceae on solid substrates (agar, sand, sterile soil, non-sterile soil). We found adaptation in cell morphology (size, cell wall thickness, plastid structure) to the different substrates used. We are currently investigating how the different substrates affect the gene expression pattern in *Zygnema circumcarinatum*.

P3. Investigating Phenolics in Response to UV Exposure in the Zygnematophycean Alga *Mesotaenium endlicherianum*

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For successful plant survival, organisms must be able to respond dynamically to rapidly changing environmental conditions. The response to abiotic challenges was crucial in the process of terrestrialisation that lead to embryophytes. Based on phylogenetic analyses, their closest streptophyte algal relatives are Zygnematophyceae, on which this poster focusses. We exposed *Mesotaenium endlicherianum* to UV-B irradiance to elucidate its response to this major abiotic stressor. Contextualised with data from embryophytes, we aim to infer the underlying molecular stress-response toolkit that was used during plant terrestrialisation. We designed two UV-B stress setups, one focussing on the early metabolomic response during an UV-B exposure of three hours followed by three hours of recovery with hourly sampling. The second setup investigates the late response after repeated UV-B exposure. For this, *Mesotaenium endlicherianum* was exposed to four hours of UV-B irradiance for three days followed by a day of recovery and sampling after 96 hours. Additional (photo)-physiological measurements were taken and revealed an expected drop in average quantum yield. Untargeted metabolite fingerprinting analysis was performed using an Ultra High Pressure Liquid Chromatography Quadruple Time Of Flight Mass Spectrometer (UHPLC Q-TOF MS) system. UV-induced metabolites such as phenylpropanoid derived compounds and purpurogallin have been identified in preliminary analyses.

P4. Ancient function and evolution of CBL/CIPK Ca2+-sensor/kinase complexes during adaptation to land

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During the conquest of land, plants developed crucial adaptations to cope with fluctuating terrestrial habitats. Ca^{2+} signalling crucially functions in these processes and involves Calcineurin B-like proteins (CBLs) and their CBL-interacting kinases (CIPKs). Coinciding with the increasing ability of plants to thrive on land, the complexity of the CBL/CIPK system also increased. This evolutionary expansion started with singular pairs still present in algae, continued with simply structured networks in bryophytes to culminate in their extant complexity in higher plants. However, the molecular drivers and mechanisms that directed the evolution of this network and its versatility remain to be identified. By comparatively studying CBL/CIPK/target modules as subject and using *M. endlicherianum* and *M. polymorpha* as model systems, we intend to address these questions. We will employ heterologous pathway reconstitution systems in yeasts, human cell lines and *A. thaliana* to delineate the functional interconnection of CBLs, CIPKs and their targets. Heterologous pathway reconstitution assays allows us to delineate the function of *M. endlicherianum* CBL/CIPK signaling complexes. Complementarily, we will use reverse genetics approaches to corroborate their physiological functions in *M. polymorpha*. In this way we intend to synthesize an evolutionary scenario for the functional diversification of CBL/CIPKs during early network formation and expansion after plant terrestrialization.

P5. From Water to Land: Unraveling TCP Transcription Factors in the Amphibious Liverwort Riccia fluitans

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Land plant evolution necessitated acquisition of adaptive traits for terrestrialization, a pivotal event in earth's history. The amphibious liverwort *Riccia fluitans* serves as an ideal model organism for investigating these traits as it grows in water and on land, developing features important for both habitats. Previous research indicates that TCP transcription factors (TFs) play a role in forming metabolic and developmental adaptations in land plants. While TCP-P and TCP-C TFs have been extensively studied in angiosperms, where they often act redundantly, their function in embryophytes remains largely unknown. Notably, *Marchantia polymorpha* and *R. fluitans* possess only one TCP gene from each clade, thereby minimizing redundancy effects. MpTCP1 was found to likely regulate redox-dependently the expression of downstream genes and thus the growth of *M. polymorpha*. Our study aims to explore the function of TCP TFs in *R. fluitans* through the generation of CRISPR/Cas9 mutants targeting each TCP gene separately. Given the monoecious nature of *R. fluitans* and the availability of only female plants, an additional dioecious Riccia species, *R. canaliculata*, is included in the research to investigate function of TCP TFs in sexual reproduction. Understanding the role of TCP TFs in Riccia and their conservation across land plants will provide further insights into the evolutionary processes and adaptive strategies employed by land plants

P6. Carbon concentrating mechanism-related protein induction and plastid rearrangement during submersion in hornworts

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Plastids play an important role in plants' responses to changing environments. In hornworts, chloroplasts can contain a RuBisCO-enriched protein matrix (pyrenoid), which enables them to perform biophysical carbon concentration at the single-cell level (CCM) – a unique feature among land plants. Based on homology to green algal CCM genes and an in-silico predicted plastid proteome, we identified a set of CCM candidate genes in *Anthoceros agrestis*. In doing so, we also assessed hornwort-specific plastid processes by label-free proteomic analysis under H_2O submersion in the pyrenoid-forming *A. agrestis* and the pyrenoid-free *A. fusiformis*. Under submersion, both species expressed CCM-like homologs, despite otherwise predominant idiosyncratic protein expression profiles. Furthermore, exposure of submersed plants to additional H_2O_2 could induce or diminish the expression of CCM-like homologs in a species-specific manner within a 48 hour timeframe. Ultrastructural analysis of plastids revealed an increase in pyrenoid-like structures and rearrangement within the first 24 hours of submersion in *A. agrestis*. In contrast, *A. fusiformis* showed no de novo birth of such pyrenoid-like structures, but an increased formation of putative plastid lipid droplets. Together, our data indicate that Anthoceros species exhibit different acclimation to submersion including the induction of pyrenoid-like structures.

P7. Molecular and biochemical characterization of cinnamic acid 4-hydroxylase in the biosynthesis of phenolic compounds in *Anthoceros agrestis*, *Chara braunii*, *Marchantia polymorpha* and *Physcomitrium patens*

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Phenolic structures are key compounds in the terrestrialization of plants, as they serve as protection against UV-light, pathogens or water loss. Their biosynthesis begins with L-phenylalanine and L-tyrosine in the phenylpropanoid pathway. In the second step, cinnamic acid 4-hydroxylase (C4H) catalyzes the hydroxylation of *t*-cinnamic acid to 4-coumaric acid (= 4-hydroxycinnamic acid). NADPH:cytochrome P450 reductase (CPR) is associated in a membrane-bound complex with C4H and is providing the electrons for this reaction. This research looks for putative C4H genes and compares enzymatic functionality of translated proteins from the hornwort *Anthoceros agrestis*, the liverwort *Marchantia polymorpha* and the bryophyte *Physcomitrium patens* and looks for putative C4H candidates in the green alga *Chara braunii*. Each hypothesized C4H gene is codon-optimized for expression in yeast and is expressed simultaneously with CPR from Coleus blumei (CbCPR). Multiple genes possibly encoding for C4H have been found in each early diverged land plant but the genome of the alga *Chara braunii* only shows vague similarities to the already characterized sequence from *Anthoceros agrestis*. Each protein translated from the land plants C4H genes has been characterized with different substrates, giving insight on their similarity. No candidate for *Chara braunii* C4H has yet been shown to catalyze the expected hydroxylation, indicating that the evolution of C4H was important for the plants' conquest of land.

P8. Evolution of the chloroplast signal recognition particle system

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The signal recognition particle (SRP) system is found in all domains of life. During the evolution of land plants, the chloroplast SRP system (cpSRP) was adapted from an ancient bacterial system and combined with novel targeting compounds to facilitate protein transport. The original SRP RNA underwent a structural change during the transition from water to land, finally leading to its loss in seed plants. Moreover, besides its function in cotranslational protein transport, the chloroplast (cp) SRP54 protein was recruited for the posttranslational protein sorting to the thylakoid membrane. There, it forms a heterodimeric complex with the plastid specific cpSRP43 protein, whose affinity increased with the preceding evolution of land plants. To further investigate the evolutionary changes of the cpSRP system, we performed an extended phylogenetic analysis of SRP components. Furthermore, we generated *Physcomitrium patens* knockout mutants of the cpSRP54 and cpSRP43 proteins to study their role in chloroplast protein transport, as the bryophytic moss represents an intermediate state of the cpSRP system and contains a combination of ancient and novel components. In addition, a transit complex formation analysis gives first insights into the cpSRP-dependent posttranslational transport of selected light harvesting chlorophyll a/b proteins in early land plants.

P9. Deciphering the role of Malectin-like RLK, MpFER in the liverwort *Marchantia polymorpha* immune system

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The cell-surface localized pattern recognition receptors (PRRs) play crucial roles in perceiving conserved molecules derived from microbes and plants in transducing immune signals. In angiosperms, the Malectin-like receptor kinase (MLR) family functions in immunity and development among various PRRs and it is responsible for perceiving RAPID ALKALINIZATION FACTOR (RALF) peptide. In the liverwort *Marchantia polymorpha*, a single MLR named MpFER has been identified, however, its specific role in immunity remains unknown. In this study, we generated and characterized Mpfer loss-of-function mutants, which fail to respond to MpRALF1 peptide treatment. We examined MpFER expression profiles by generating MpFER promoter::GUS reporter lines and explored the network of proteins interacting with MpFER using a miniTurbo-based interactome approach. Our findings indicate that potential MpFER interactors are involved in multiple and diverse processes, including immune signalling, metabolic process, and cell growth. By comparing the transcriptomes of Marchantia upon MpRALF1 and chitin treatments, we observed that genes induced by MpRALF1 are involved in the defence responses. Overall, our study demonstrates the conservation of FER-RALF module in the liverwort Marchantia and its contribution to plant immunity.

P10. Insights from *Mesotaenium endlicherianum* in the terrestrial plant evolution of drought response

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The response to drought stress in land plants has been extensively studied, with key findings including stomatal closure, reduced photosynthesis, increased production of late embryogenesis abundant proteins and aquaporins, and the generation of reactive oxygen species. However, little is known about how the alga *Mesotaenium endlicherianum*, which belongs to the sister-clade of embryophyta, responds to osmotic and salt stress. Recently conducted multi-omics experiments shed light on the various drought-related responses exhibited by this single-celled alga. Most of these responses are conserved among both Embryophyta and Zygnematophyceae species and include the increase of ROS-scavenger proteins and Early Light-Inducible Proteins (ELIP). The findings also provide an intriguing insight into a NaCl-inducible cell wall response and reveal new, unidentified proteins associated with drought stress.

P11. DiversiPHY and conquer — Diversification of phytochromes during plant terrestrialisation

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Phytochromes (PHYs) are photoreceptors in plants that play a key role in the adaptation to the (light) environment. Canonical plant PHYs originated in a common ancestor of extant streptophytes. Independent gene duplications resulted in PHY families in seed plants, ferns, and mosses. Functional diversification into PHYs specifically sensing red (R) or far-red (FR) light is well documented for seed plants but has not been explored for other land plants. In the first funding period of MAdLand, we have addressed this question for moss PHYs, but functional diversification of fern PHYs has not yet been investigated. Furthermore, molecular determinants underlying functional diversification into either R or FR light-specific PHYs are still largely unknown and it is still unclear if factors that determine the wavelength-specificity of PHYs are conserved in mosses, ferns, and seed plants. We hypothesise that FHY1 and PIFs are important for wavelength-specific PHY responses. In future work, we want to address these questions and identify the molecular basis for PHY functional diversification.

P12. Project outline: Evolution of lipid droplet-associated proteins and their role in drought resistance

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Drought is a prime stressor that plants had to overcome in their conquer of land. Most land plants are able to adapt to drought on the cellular level. Furthermore, they are able to produce desiccation tolerant cells and tissues as part of their reproductive cycle such as seeds, pollen and spores. However, many streptophyte algae are also displaying high stress resilience. Common to both drought resistance and desiccation tolerance is the accumulation of neutral lipids, foremost triacylglycerol, in cytosolic lipid droplets (LDs) also referred to as oil bodies, lipid bodies or oleosomes. Several protein families are known to localize to LDs and two of them caleosin and lipid droplet associated protein are associated with drought stress responses and possibly evolved this function in streptophyte algae. The goal of the research project is to find out if the accumulation of neutral lipids in LDs is a universal drought stress and desiccation response in the clade of Streptophyta by investigating the five species. Furthermore, we want to collect evidence that LD-associated proteins are important to cope with this stress, and evolved this function already in streptophyte algae. Overall, this data would support the hypothesis that the accumulation of LDs and its associated proteins during drought was one of the prerequisites for the conquer of land.

P13. Phylogenomic insights into the first filamentous streptophyte

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Streptophytes are renowned for encompassing the entire spectrum of land plants. Amidst this rich diversity of embryophytes, a group of freshwater and terrestrial algae holds crucial information about the origin of key land plant traits. Among these algae, the Klebsormidiophyceae stand out for their exceptional adaptability to diverse environments, ranging from the Atacama Desert to the Antarctic. Klebsormidiophyceae can display filamentous body plans and are resilient colonizers of terrestrial habitats. However, the absence of a robust phylogenetic framework for the Klebsormidiophyceae has hindered our understanding of the evolutionary history of these essential traits. We conducted a phylogenomic analysis utilizing advanced models to address this knowledge gap. We sequenced 25 new transcriptomes and integrated them with 14 previously published genomic and transcriptomic datasets. Analyzing 199 loci, we successfully established a novel phylogenetic structure. Our findings unveiled six distinct genera within the Klebsormidiophyceae: Streptofilum, Interfilum, Klebsormidium, Entransia, Hormidiella, and Streptocarsina. Expanding on this phylogenetic structure, we conducted ancestral state reconstruction with a specific focus on the emergence of the filamentous trait within streptophytes in Klebsormidiophyceae.

P14. Phylogenomics of Zygnematales

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Zygnematophyceae are recently known to be the closest ancestors of Embryophyta (land plants) and play a significant role in understanding plant evolution. Initially, they were overlooked due to their relatively simple body plan. Zygnematophyceae exhibit high species richness and have a wide distribution, commonly found in nearly all freshwater habitats. Some of them were described more than 100 years ago. Despite their importance and frequent occurrence, their internal relationships remain mostly unknown. This study builds upon recent discoveries from Hess et al. (2022) and aims to contribute to further phylogenomic analyses by using transcriptomic data, specifically focused on testing hidden genetic diversity and strain inconsistency within Zygnematophyceae. The primary focus of this research is on the order Zygnematales.

P15. Characterisation of regulatory elements for the control of gene expression governing developmental adaptations and acclimation responses in charophycean green algae

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We investigate the evolution of gene expression networks, regulatory mechanisms and promoter architectures, with a focus on the streptophyte green algae *Chara braunii*. Thus far, we have established the reliable cultivation of *Chara braunii*, protocols for the extraction of total RNA, and the generation of cDNA and sRNA libraries. Based on the analysis of several Chara transcriptome datasets, we provide first insights into stress-related gene regulation for salinity, illumination and carbon availability. Furthermore, we have begun to look into the developmental genetics of *Chara braunii* through nodal cell transcriptome datasets (1). Additionally, we investigate the transcription start sites and 5'UTR regulatory elements of genes encoding crucial photosynthesis and RNA interference proteins and provide data on suspected RNA editing events, which are currently under investigation and experimental validation. We present data on the functions of divergently regulated genes, mRNA architectures, the actual RNA accumulation in vivo as well as proteins affiliated with the RNA interference machinery and photosynthetic apparatus. (1) Heß D., Holzhausen A., Hess W.R. (2023) Insight into the central and nodal cells transcriptome of the streptophyte green alga *Chara braunii*. BIORXIV (preprint). DOI: 10.1101/2023.02.12.528195.

P16. MIRNA genes differentially expressed in vegetative and reproductive organs of *Marchantia polymorpha* – insights into their structure, expression pattern and function

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Throughout the course of plant's life cycle, microRNAs coordinate gene expression in a robust and dynamic manner influencing numerous plant developmental processes. *Marchantia polymorpha* is a classical model to study conserved and diversified plant developmental processes due to its significant phylogenetic position. To date, six conserved and three unique miRNA-target mRNA modules have been functionally studied in Marchantia. However, comparative research focusing on similar functions in liverworts and other basal plants is crucial to acquire a thorough knowledge of the role of additional miRNAs and their targets in regulatory networks controlling Marchantia development. We chose to study liverwort-specific (MpmiR11737a, MpmiR11865*) and Marchantia-specific (MpmiR11796, MpmiR11887) miRNAs as they showed differential expression in Marchantia vegetative and reproductive organs. We found that MpmiR11737a, MpmiR11865* and MpmiR11796 are encoded by independent transcriptional units and show primary-miRNA abundance and processing in different organs to learn possible layers of miRNA transcriptional and co/posttranscriptional regulation. We present degradome data of selected mRNA targets and found a negative correlation in the expression pattern between miRNA-target mRNA levels suggesting their roles as post-transcriptional regulators of mRNA targets necessary for Marchantia development and reproduction. Additionally, the phenotype of KO mutant plants for microRNAs will be shown.

P17. Divide and conquer: Evolutionary adaptations of the plant cytoskeleton during cell division

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The colonization of terrestrial habitats by ancestral streptophyte algae, followed by the rise of land plants was accompanied by a switch in the mode of cell division from a cleavage-like to an inside-out mechanisms, in which new cell walls are inserted at the cell center and expand centrifugally to fuse with the maternal cell wall at a predetermined cortical region. The switch in cell division coincides with the evolution of two plant-specific mitotic cytoskeleton arrays, the preprophase band and phragmoplast. The molecular mechanisms underlying these adaptations, however, are still enigmatic. Using a combination of phylogenetic, molecular, and cell biological analyses, we provide first evidence for emergence of plant-specific IQ67-Domain proteins in the streptophyte lineage and evolutionarily conserved functions in regulation of mitotic microtubule arrays, potentially by providing scaffolds that aided the rewiring and neo-functionalization of protein-protein-interaction networks.

P18. The role of a MYB-like transcription factor family that originated in streptophytic algae

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Previously, we identified a family of MYB-like transcription factors coined KUODA (Chinese for "to enlarge"), based on the observation that its first member promotes cell expansion in *Arabidopsis thaliana*. Members of the KUA MYB-related family contain a MYB-SHAQKYF domain and a K/M/RLFGV domain that is important for transcriptional repression. Furthermore, some members contain an additional EAR motif. Here we use a comparative approach to understand the role of KUA transcription factors during the water to land transition. We present here a detailed phylogenetic overview of the KUA family, tracing back its origins in streptophytic algae. In addition, preliminary analysis of the *Marchantia polymorpha* KUA1 in vivo and in vivo is presented. Ultimately we are interested in unraveling the evolution of the gene regulatory network controlled by KUA transcription factors, and its utilization in the conquest of land and/or alternation of generations.

P18. Evolutionary impact of small RNA-dependent gene expression in bryophytes during the molecular adaptation for life on land

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One important molecular adaption to life on land was the development of fast and flexible regulation of gene expression via microRNAs (miRNAs). This type of expression control enabled plants to adapt to the multiple changes in the new environment, including abiotic stress. We identified a miRNA-controlled gene contributing to the salt tolerance of *Physcomitrium patens*. This gene encodes a membrane protein homologous to the three Flotillin (FLOT) variants of *Arabidopsis thaliana.* Our current research shows that the single *P. patens* FLOT protein is located in the chloroplasts. Moreover, its expression level is strongly correlated with the degree of salinity tolerance, the pigmentation of protonema cells and the formation of vegetative diaspore-like cells. Furthermore, we generated CRISPR/Cas-mediated mutant lines for all four encoded DICER-LIKE proteins in *Marchantia polymorpha* and observed severe phenotypic alterations in these lines, including developmental variations and altered responses to phytohormones and salinity. Further mRNA- and sRNA-sequencing in these mutant lines will help us to understand the evolution of DCL-based sRNA-dependent gene regulatory networks in ancient land plants.