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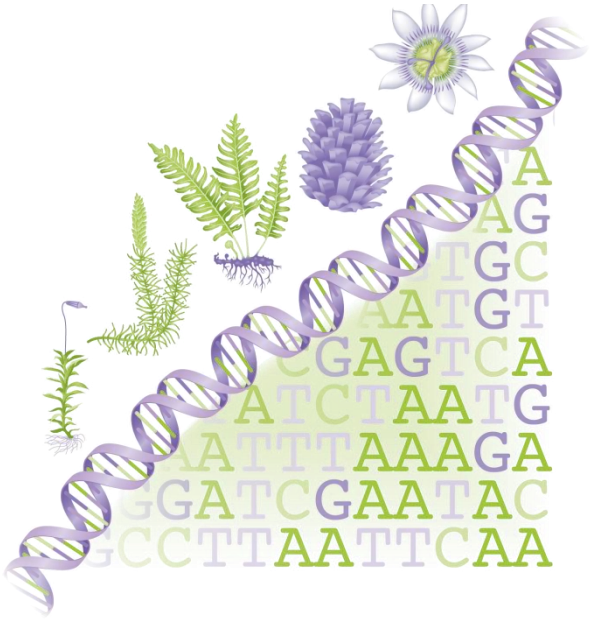

ABSTRACT BOOK

FOR5098 *ICIPS*
(*Innovation and Coevolution in Plant Sexual Reproduction*)
Symposium

Evolution of Plant Reproduction

25.-28.03.2025
Elisabeth-Schiemann lecture hall
Königin-Luise-Strasse 12-16
14195 Berlin - Germany

Deutsche Botanische Gesellschaft
CLF Plant Climatics
The Company of Biologists
Novogene

CONTENT:

International *ICIPS* Symposium: *Evolution of Plant Reproduction*
Preliminary Schedule

Tuesday 25.03.2025				Wednesday 26.03.2025				Thursday 27.03.2025				Friday 28.03.2025			
Start	End	Duration	Speaker	Start	End	Duration	Speaker	Start	End	Duration	Speaker	Start	End	Duration	Speaker
				Session 2: Evolution of multicellular embryos & endosperm				*Session 5: Evolution of fertilization mechanisms in plants*				*Session 7: Co-evolution of signaling systems and GRN in development*			
				8:30	9:00	30	**Gwyneth Ingram**	8:30	9:00	30	**Tetsuya Higashiyama**	8:30	9:00	30	**Sebastian Schornack**
				9:00	9:20	20	**Rency Pulickal**	9:00	9:20	20	**Sophie Tiedemann**	9:00	9:20	20	**Melanie Trupp**
				9:20	9:40	20	**Daniela Barro-Trastov**	9:20	9:40	20	**Saurabh Joshi**	9:20	9:40	20	**Ueli Grossniklaus**
				9:40	10:00	20	**Martin Bayer**	9:40	10:00	20	**Yukiho Toyama**	9:40	10:00	20	**Margot Smit**
				10:00	10:20	20	*Coffee break*	10:00	10:20	20	*Coffee break*	10:00	10:20	20	*Coffee break*
				10:20	10:40	20	**Terezie Mandakova**	10:20	10:40	20	**Joakim Palovaara**	10:20	10:40	20	**Feng Zhao**
				10:40	11:10	30	**Claudia Köhler**	10:40	11:10	30	**Meng-xiang Sun**	10:40	11:10	30	**Kerstin Kaufmann**
				Session 3: Evolution of ovules & seeds				*Session 6: From ROS to hormones*				*Session 8: Bioinformatics solutions to harness the EvoDevo Data Chaos*			
12:00	14:00		*Registration*	11:10	11:40	30	**William Friedman**	11:10	11:40	30	**Hugues Renault**	11:10	11:35	25	**Oliver Rupp**
				11:40	12:10	30	**Lucia Colombo**	11:40	12:00	20	**Nora Gutsche**	11:35	12:00	25	**Clemens Rössner**
				12:10	13:30	90	*Lunch break*	12:00	13:30	90	*Lunch break*	12:00	12:30	30	*Coffee* Break *(lunch bags)*
14:00	14:15	15	*Welcome*	13:30	13:50	20	**Véronique Hugouvieux**	13:30	13:50	20	**Tom Dierschke**	12:30	12:55	25	**Nicholas Desnoyer**
Session 1: Innovation of pollen & their reception/rejection systems				13:50	11:40	20	**Kay Schneitz**	13:50	14:10	20	**Nicola Babolin**	12:55	13:20	25	**Ana Florez-Rueda**
14:15	14:45	30	**Noni Franklin-Tong**	14:10	14:30	20	**Robin Schulz**	14:10	14:30	20	**Emanuela Talarico**	13:20	13:55	35	**James Clark**
14:45	15:05	20	**Matteo Barois**	14:30	15:00	30	**Andrew Plackett**	14:30	15:00	30	**Isabel Monte**	14:00	14:15	15	*Poster & Talk prizes*
15:05	15:25	20	**Silvia Moschin**	*Session 4: Evolution of carpels & fruits*				15:00	18:00	3h	*Guided Tour:* - Herbarium collection - Dahlem Seed Bank Center - Botanical Garden	14:15	14:30	15	*Closing remarks*
15:25	15:45	20	*Coffee break*	15:00	15:30	30	**Madelaine Bartlett**								
15:45	16:05	20	**Xia Chen**	15:30	15:50	20	*Coffee break*					Invited Speaker Talk (25 min + 5 min questions)			
16:05	16:25	20	**Daniel Bouyer**	15:50	16:10	20	**Dmitry Sokoloff**					Selected Speaker Talk (15 min + 5 min questions)			
16:25	16:45	20	**Marie Monniaux**	16:10	16:30	20	**Julian Garrecht**					Flash Talks (5 min each / 3 slides max.)			
16:45	18:00	75	Poster session 1	16:30	16:50	20	**Aline Janeau**					Poster sessions			
				16:50	17:10	20	**Rainer Melzer**								
				17:10	17:40	30	**Charlie Scutt**								
18:30	20:00	90	*Reception* Landhaus restaurant in the Botanical Garden	17:40	18:10	30	Flash Talks	18:30	21:00		*Conference Dinner* Landhaus restaurant in the Botanical Garden				
				18:10	19:00	50	Poster session 2								

Poster List

Tuesday, 25.03.2025 & Wednesday, 26.03.2025		
N°	**Name**	**Title**
1	Dilsher Singh **Kulaar** *	*Role of DNA methylation in asexual endosperm formation*
2	Yichun **Qiu** *	*Duplication of MADS-box genes in land plants empowered the functional divergence between sporophytes, gametophytes and endosperm*
3	Bridget **Bickner** *	*Evolution and development of ovule packaging variation in the Phlox genus*
4	Camille **Salaün** *	*Genetic determinants of autonomous endosperm formation in Arabidopsis*
5	Gerardo **Del Toro** *	*Maternal control of RNA decay safeguards embryo development*
6	Micha Frederick **Keßler** *	*CoExPlore: Interactive comparative analysis of co-expression networks*
7	Chan **Liu**	*Mechanical control of germ cell specification in Arabidopsis anthers*
8	Xiaocai **Xu**	*Dissecting transcriptional regulation of endosperm cellularization at the single nucleus level*
9	Kai **Wang**	*Identifying the origin of Pol4-dependent small RNAs in the endosperm*
10	Ana Catarina **Silva**	*Identifying genetic determinants of apomixis in dandelion T. officinale*
11	Haruto **Yahiro**	*Post-pollination reproductive barriers driving speciation: insights from Torenia crustacea*
12	Pavla **Novotná**	*How to elucidate dosage compensation and parental conflict in Silene latifolia*
13	Sinah Tabea **Ehlert**	*Unravelling the influence of temperature on the switch from sexual to apomictic seeds through phenotypic plasticity*
14	Petra **Šarhanová**	*The secrets of apomictic seeds: more sperms, less troubles.*
15	Chi-Ying **Hsueh**	*Brassinosteroid-mediated environmental plasticity of seed development*
16	Matteo **Caroulle**	*The carpel: A problem child we fail to grasp*
17	Siwei **Pang**	*Evolutionary insights into plant reproduction: the role of JAGGED-like zinc finger transcription factors*
18	Xuecheng **Zhang**	*A female in vivo haploid-induction system via mutagenesis of egg cell-specific peptidases*
19	Hannah **Lepper**	*The role of trehalose 6-phosphate and TREHALOSE-6-PHOSPHATE SYNTASE in the evolution of land plants*
20	Sophie **De Vries**	*Evolution of the miR156/529-SPL network in land plants*
21	Ellen Sigourney **Lorberg**	*Evolution of reproductive coordination in Azolla*
22	Nicholas **Desnoyer**	*Open Flower: A blooming model for teaching, outreach and research*
23	Henri **Dümpelmann**	*Comparing expression of orthologous genes between different plants using GenXBrowser*
24	Julian **Ingelfinger** / Stefanie **Müller-Schüssele**	*The secreted redox sensor roGFP2-Orp1 reveals oxidative dynamics in the plant apoplast*

* Will participate in the Flash Talks Session on Wednesday afternoon

Abstracts of Oral Presentations

SESSION 1
Innovation of Pollen and their Reception/Rejection Systems
Chair: Thomas Dresselhaus (Regensburg)

Self-incompatibility: diverse mechanisms involved in allorecognition and prevention of self-fertilization

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The ability to discriminate between self and non-self (allorecognition) is important to most multicellular organisms. Complex pollen-pistil (male-female) interactions play a decisive role in determining reproductive success following pollination. Self-incompatibility (SI) is probably the most important mechanism utilised by plants to prevent inbreeding. SI is a very ancient phenomenon, which has persisted in lineages for at least ~90 million years. It is used by ~50% of higher plant species and because it increases levels of genetic variation, it is thought to be one of the major reasons for the success of angiosperms. During pollination, "self" (incompatible) pollen is discriminated from compatible pollen, and rejected. Three mechanistically distinct homomorphic SI systems have been identified and key features of how they act to reject self-pollen have been elucidated at the molecular level. They employ different S-determinants and mechanisms to prevent self-fertilization. The presence of these different SI systems provides strong evidence that SI has evolved independently several times. I will review the main characteristics of these well characterized SI systems but emphasize that there are probably many more SI systems that have not been identified yet. My research career has focused on the SI system in Papaver rhoeas (poppy), which has turned out to be an outstanding model system for cell-cell recognition/signalling. I will provide an overview of what we know about the mechanisms involved in regulating this SI system, which involves the triggering of a signalling network involving increases in cytosolic Ca2+ and reactive oxygen species, cytosolic acidification, a decrease in ATP and changes in actin organisation, leading to activation of caspase-like proteases and PCD. Identifying the diversity of SI systems and how they evolved is a challenge for the future.

Transcriptional regulation of dominance at the self-incompatibility locus in *Arabidopsis*

Matteo Barois, Rita A. Batista, Pierre Baduel, Etienne Delannoy, Vincent Castric
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University of Lille

Self-incompatibility (SI) mechanisms in hermaphroditic flowering plants serve as crucial barriers to self-fertilization. In the Brassicaceae, the locus governing SI

displays remarkable diversity, featuring numerous distinct alleles retained over long evolutionary times and organized in a complex dominance hierarchy. Under this hierarchy, the gene controlling SI specificity in pollen exhibits monoallelic expression in heterozygote individuals. This is achieved through the action of small RNAs (sRNAs) produced by precursors acting as ”dominance modifiers” resembling miRNAs but compatible with multiple gene silencing pathways. Single sRNA precursor undergoes extensive processing, generating hundreds of sRNA molecules with varying sizes, abundance levels, and ARGONAUTE loading preferences. To study this gene silencing phenomenon, we established a reverse genetic approach in engineered Arabidopsis thaliana lines expressing components of the A. halleri SI system and observed that the transcriptional repression is independent of the canonical RNA-directed DNA Methylation pathway (RdDM). We developed a single-molecule transcript capture protocol, and remarkably we observed that the sRNAs seem to target the transcription start site, possibly indicating an interference with the transcriptional machinery. Overall, the question of the mechanisms by which this repression occurs remains open, especially regarding the role of DNA methylation on target sequences. Unraveling these silencing mechanisms will offer insights into the mechanisms by which dominance/recessivity interactions can evolve.

Unravelling pollen-ovule cross-talk in *Ginkgo biloba*: insights into gymnosperm reproductive biology

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Four of the five major lineages of seed plants are gymnosperms; therefore, studying their reproductive biology is crucial to better understand the evolution of reproductive mechanisms in seed plants. Our primary focus has been on the gymnosperm *Ginkgo biloba*. We have characterized ovule and microsporophyll development, identifying key stages. We have found that pollination is essential for ovule development to progress; therefore, we are now concentrating on investigating the key factors involved in ovule-pollen cross-talk. First, we aim to study in detail the ultrastructure and composition of the pollen coat to identify specific Ginkgo pollen determinants, their location, and the timing of their exposure. We hypothesize that, upon reaching the pollination drop extruded by the ovule, pollen grains hydrate, undergo significant shape changes, and expose specific areas of the pollen coat that may contain signaling molecules involved in ovule-pollen recognition. Subsequently, pollen enters the ovule through the pollen chamber, where it is presumed to undergo intense metabolic activation before germination. Additional signalling molecules may be exposed at the intine upon germination, further contributing to ovule-pollen cross-talk. In Ginkgo, pollen tube growth is haustorial: numerous small branches grow, penetrating between the cells of the nucellus and extracting nutrients for several months. Meanwhile, the female gametophyte develops within the ovule, ultimately forming the seed’s storage tissue. We believe that the development of both gametophytes is closely interconnected and interdependent. Our aim is to identify the signals - from both sides - involved in this communication throughout ovule development, from pollination to fertilization.

Functional analysis of RALF gene family members in maize and their evolution

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The evolution of land plants has resulted in a wide range of complexity, from the earliest algal ancestors, through the nonvascular bryophytes and vascular seedless plants, to the complex gymnosperms and angiosperms of today. Compared to ancient plants, gametophytic communication systems in reproductive organs of angiosperms are more complex due to the evolution of specialized sexual organs allowing reproduction in dry environments. Various reports indicate that RAPID ALKALINIZATION FACTORs (RALFs) play key roles in short range gametophyte cross-talk during pollen hydration, germination, tube growth and reception in Arabidopsis. Also in grasses like maize, we recently found that ZmRALF2/3 are also involved in regulating cell wall integrity during pollen tube growth in maize. Phylogenetic and expression analysis of the maize RALF protein family have shown that different members are highly and specifically expressed in pollen grains and during tube growth in maize. ZmRALF2/3 belong to Clade I RALFs, which include the typical RALFs found in ancient plants. However, ZmRALF1/5 belonging to Clade III generate about 50% RALF transcripts in maize pollen tubes. Unlike typical RALFs, ZmRALF1/5 lack the conserved S1P protease cleavage sites, YISY domain and YY domain, which are important for interaction with LRR-extensin cell wall proteins. An overview about the evolution of the RALF family and new function(s) of ZmRALF1/5 and their origin are important questions that will be addressed.

Molecular Evolution of epigenetically regulated pollen genes

Bertrand Huguenin-Bizot, Annabelle Haudry, Fiamma Bunello, Emanuele De Paoli, Daniel Bouyer
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DNA methylation is important for genome maintenance and gene regulation in animals and plants. In both mammals and flowering plants, this epigenetic mark is reprogrammed during the reproductive phase and this process is required for the activation of genes important for pollen function and male fertility. DNA demethylation in pollen counteracts heterochromatic silencing mechanisms established in the vegetative plant and as a consequence, male fertility genes share epigenetic characteristics of transposable elements. However, the DNA methylation signature of these genes varies in nature, questioning the gene regulatory requirement of this epigenetic mark. Here, we explore the impact of DNA methylation on the molecular evolution of epigenetically regulated pollen genes and will discuss the consequences for their selection regimes.

Evolving as a couple: diversification of the ligand-receptor interaction for self-incompatibility

Marie Monniaux, Shin-Yi Yu, Céline Poux, Maxime Chantreau, Xavier Vekemans, Julie Bouckaert and Vincent Castric
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A large fraction of flowering plants, although being hermaphrodites, are prevented to self by a process called self-incompatibility (SI), which avoids the deleterious effects

of inbreeding depression. In Brassicaceae, SI is encoded in the S-locus that contains two tightly linked genes: the SRK gene encoding a receptor kinase expressed in stigmatic papillae cells, and the SCR gene encoding a small protein displayed at the surface of the pollen. Upon interaction of SRK and SCR partners, the pollen is recognized as self and pollen germination is blocked. In a given species, there are typically 60-100 different S-haplotypes, each encoding variants of the SRK and SCR proteins that interact in a specific manner, ensuring that selfing is avoided and that outcrossing happens with all other compatible mates. How such a vast set of specific ligand-receptor interactions has emerged, is maintained and how a new couple of interactors can evolve without disrupting the fitness of the plant are puzzling questions. To explore these questions, we have focused on a set of closely related S-haplotypes and reconstructed their putative ancestral protein sequences. This has revealed, for the SRK receptor, a process of asymmetrical diversification, with one SRK allele retaining the same specificity as the ancestor, and the other allele evolving a new specificity. We are now exploring the evolution of the highly divergent SCR ligand by ancestral protein reconstruction, and evolution of the SRK-SCR complex structure by experimental and modeling approaches.

SESSION 2
Evolution of Multicellular Embryos and Endosperm
Chair: Duarte Figueiredo (Potsdam)

Embryo-endosperm communication in developing seeds: Establishing the boundaries of an ambiguous relationship.

Audrey Creff, Nicolas Doll, Camille Salaün, Julien Larive, Vincent Bayle, Duarte D. Figueiredo, Benoit Landrein and Gwyneth Ingram
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In angiosperms, the double fertilization of ovules gives rise to two distinct organisms; the embryo and the endosperm. Although genetically similar, their developmental trajectories within the seed are dramatically different. The embryo develops into a “baby plant”, differentiating tissues and meristems that will permit its ultimate germination and growth into a new individual. In contrast the endosperm appears to play a “nursing” role, transferring nutrients from the mother plant to the embryo, and ultimately dying. To what extent the development of these two organisms is interdependent remains controversial. I will focus on peptide mediated dialogues between the embryo and the endosperm. I will discuss advances in our research showing that the endosperm, in addition to its nursing role, provides developmental cues to the embryo. I will then go on to show new data suggesting that, contrary to the currently accepted view, endosperm development is also profoundly influenced by the embryo. Our findings shed light on the importance of molecular dialogues between these two “sibling” organisms, and raise interesting questions about the control of molecular diffusion in the apoplasts of reproductive structures.

Paternally derived brassinosteroid effectors as regulators of ab initio cellular endosperm formation

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Angiosperm seeds consist of three genetically distinct structures: embryo, endosperm, and seed coat. Based on its cellularization pattern, there are three types of endosperm development: nuclear, cellular, and helobial. Recent studies from our group in *Arabidopsis thaliana*, which has a nuclear-type endosperm, showed that the phytohormone brassinosteroids (BRs) are synthesized in the seed coat and non-cell autonomously regulate endosperm development. But so far there are no detailed studies on the hormonal regulation of cellular endosperm development. To investigate this, we used tomato (*Solanum lycopersicum*) as a model organism, as tomato endosperms are ab initio cellular. Our results reveal that exogenous BRs accelerate endosperm proliferation in tomato, indicating a positive regulatory role for BRs in cellular endosperm development. To further validate this, we knocked out SlBZR1, which encodes a transcription factor involved in the BR signaling pathway, and which is paternally expressed in the tomato endosperm. Indeed, introduction of the slbzr1 mutation via the pollen leads to reduced seed size and endosperm expansion. Surprisingly, the paternal introduction of slbzr1 also results in delays in fruit ripening, implicating paternal factors as regulators of fruit development. Moreover, we confirmed that SlBZR1 is expressed in the tomato endosperm and in the seed coat, unlike what happens in Arabidopsis, where its homologue is only expressed in the seed sporophytic tissues. Furthermore, we observed that BZR1 is paternally imprinted in endosperm of early diverging angiosperms, which also produce ab initio cellular endosperms. Our work thus implicates BR effectors as paternal signals regulating cellular endosperm formation.

Unravelling the molecular basis of endosperm evolution

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The endosperm, a tissue found in the seeds of flowering plants, plays a crucial role in the development and nourishment of the plant embryo. Proper endosperm development is essential for the successful growth and germination of seeds, ensuring the survival and dispersal of the next generation. Moreover, due to its nutritional richness, the endosperm serves as a primary calorie source for humans and animals. Understanding the molecular mechanisms behind endosperm development and evolution could shed light on essential plant biological processes and potentially leads to the exploration of strategies aimed at enhancing crop productivity. Recent research conducted in our group supports the hypothesis that Type I MADS-box transcription factors are involved in the emergence of the endosperm in flowering plants. Particularly noteworthy is PHERES1 (PHE1), a master regulator of key endosperm development genes. Our findings reveal that DNA-binding motifs targeted by PHE1 overlap with Helitrons, a specific type of transposable elements. This observation suggests that the spreading of PHE1 binding sites throughout the genome via transposition facilitated the recruitment of endosperm regulators into a unified transcriptional network. To test this model, we aim at identifying PHE1-like genes in water lily, an early diverging angiosperm with rudimentary endosperm, and in the

monocot maize, characterized by plentiful endosperm. Additionally, we intend to examine the potential overlaps between Helitrons and the binding motifs recognized by those PHE1-like genes. We found that PHE1-like genes are active in the maize endosperm, consistent with findings in Arabidopsis. Moreover, in maize, some PHE1-like targets include genes with functions in endosperm proliferation, aligning with observations in *Arabidopsis* as well. Overall, our findings highlight evolutionary conserved regulators of endosperm development, which may serve as valuable breeding targets.

MAP kinase signaling in cell polarity – how parental signals shape the plant embryo

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Breaking symmetry by asymmetric cell divisions is essential for establishing different cell identities in multi-cellular development. In land plants, cell types are established in a position-dependent manner. Therefore, cell-cell signaling by receptor kinase/MAP kinase signaling pathways is a reoccurring mechanism in polarizing cells and establishing different cell identities in the daughter cells of asymmetric cell divisions. We are using the early *Arabidopsis* embryo as system to study initial events during cell polarization by the ERECTA-YODA signaling pathway – a prototype receptor kinase/MAP kinase signaling pathway. By comparing its function in the embryo with its role in planar patterning of the leaf epidermis, we identified a core pathway and context-specific modifications. We present new data on the impact of polar YODA activation on early embryonic patterning and shed light on the mechanism and evolution of distinct modes of YDA activation in the zygote on a molecular and structural level. We furthermore discuss possible benefits of different modes of YDA activation and their distinct parent-of-origin effects.

The origin and evolution of apomixis-associated chromosomes in *Boechera*

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Masaryk University

The genus *Boechera* (Brassicaceae) consists of approximately 480 genetically distinct taxa, primarily distributed across North America. With a basic chromosome number of x = 7, this genus includes diploid sexuals (2n = 14), diploid apomicts (2n = 14, 15), and triploid apomicts (2n = 21, 22). Its remarkable morphological diversity arises from frequent hybridization among diploid species, resulting in the formation of diploid apomictic hybrids, while triploids emerge independently through hybridization between sexual and apomictic diploids. Apomictic *Boechera* plants are uniquely characterized by the presence of two heterochromatic, apomixis-associated chromosomes, Het and Del. We have successfully generated telomere-to-telomere genome assemblies for several sexual and apomictic *Boechera* accessions, achieving detailed resolution of centromeres and heterochromatic regions. These genomic analyses were complemented by cytogenetic and epigenetic studies, revealing significant links between hybridization, chromosome reorganization, and the evolution of apomixis-associated chromosomes. Notably, our findings provide critical insights into the structural and functional dynamics of Het and Del, shedding light on their pivotal roles in regulating apomixis. Through the integration of advanced

sequencing technologies and cytogenomic tools, Boechera has been firmly established as a premier model system for studying genomic changes associated with apomixis. Our results not only deepen the understanding of the genomic impact of apomixis but also contribute to the development of refined hypotheses on the molecular mechanisms underpinning its expression. This work represents a significant advancement in elucidating the cytogenetic foundations of apomixis, with far-reaching implications for plant reproductive biology and evolutionary genomics.This work was supported by the Czech Science Foundation (grant number 24-11371S).

Transposon-driven endosperm evolution and its consequences for angiosperm diversity

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Transposable elements (transposons) are mobile genetic sequences that can move within the genome, driving genetic innovation over evolutionary time. I will discuss how transposon activity has influenced the evolution of the endosperm, a seed tissue that supports embryo growth similar to the mammalian placenta. The endosperm, unique to flowering plants, forms when one sperm cell fuses with the central cell, while another fuses with the egg to form the embryo. Our research identified type I MADS-box transcription factors as key regulators of endosperm development. Strikingly, the binding sites for these factors are located within Helitron transposons, suggesting that transposons dispersed these regulatory sites across the genome. This highlights the essential role of transposons in endosperm evolution, shaping regulatory networks and epigenetic landscapes. These networks, however, become disrupted during interspecies hybridizations, leading to endosperm developmental failure and seed arrest, which in turn promote speciation in flowering plants. Using the *Capsella* genus as a model, we found that hybridizations between closely related *Capsella* species result in endosperm defects linked to chromosome condensation failures and loss of DNA methylation. These defects can be mitigated by increasing the ploidy of the maternal parent, indicating that a dosage-sensitive component in the maternal genome plays a critical role in hybridization success. We identified maternally-produced small RNAs, which guide DNA methylation in the endosperm, as this key component. An imbalance in these small RNAs between the parental genomes leads to improper gene regulation and endosperm arrest, providing new insights into the genetic basis of hybridization barriers.

SESSION 3
Evolution of Ovules and Seeds
Chair: Günter Theissen (Jena)

Angiosperm seeds are a mess!

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With five separate organismal and genetic entities (male gametophyte, female gametophyte, maternal sporophyte, embryo, and endosperm) interacting in every seed,

it is amazing that flowering plants have made it this far. Indeed, the angiosperm seed is a veritable arena of conflict and cooperation among these five genetically and ontogenetically distinct organisms. How did angiosperms end up with mothers and fathers arguing over endosperm-provisioning while sibling endosperms and embryos practice the noble art of altruism? I will examine the evolutionary origin (and potential homology) of a genetically biparental embryo-nourishing tissue (endosperm) in the common ancestor of flowering plants, as well as the evolutionary and developmental consequences of the insertion of a paternal genome into the "business" of maternal embryo-nourishing strategy. As it turns out, packing the whole family into a seed can be messy, but also can lead to great things (like angiosperms).

SPOROCYTELESS and _MADS_-domain factors are required for differentiation of megaspore mother cell (MMC)

Alex Cavalleri, Chiara Astori, Silvia Manrique, Cezary Smaczniak, Greta Bruzzaniti, Chiara Mizzotti, Mattia Spanò, Alessandro Ruiu, Andrea Movilli, Kerstin Kaufmann, Lucia Colombo
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In angiosperms, the megaspore mother cell (MMC) undergoes differentiation within the ovule. The MMC undergoes meiotic division to produce a tetrad of haploid megaspores, of which one will develop into the functional megaspore (FM) and three will degenerate. The MMC undergoes meiotic division to produce a tetrad of haploid megaspores, of which one will develop into the functional megaspore (FM) and three will degenerate. After undergoing three rounds of mitosis, the FM develops into a female gametophyte. NOZZLE/SPOROCYTELESS (NZZ/SPL) is the primary regulator of MMC differentiation in Arabidopsis thaliana. Mutations in the SPL gene result in complete female and male sterility due to the absence of both the MMC and the PMC (pollen mother cell) (Schiefthaler et al., 1999; Yang et al., 1999). Although SPL does not directly bind DNA, it has been proposed that it controls target genes by interacting with transcription factors like TPC and by recruiting corepressors such as TPL and TPR to repress the transcription of target genes (Chen et al., 2014; Wei et al., 2015). Despite SPOROCYTELESS's importance, little is known about its target genes and molecular interactions. We have concentrated on examining SPL interactors in ovules using CO-IP/MS. We have used RNA-seq and CHIP to find direct SPL targets, and as a result, we have proposed a possible downstream network.

SEPALLATA driven _MADS_ complex formation is required for ovule and seed development in _Arabidopsis_

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A fundamental question in plant biology is how flowering plants evolved to dominate the terrestrial landscape, outcompeting their seed plant sister clade, the gymnosperms. One hypothesis is that gene duplication events in the MADS family, key regulators of reproductive organ development in all seed plants, allowed the development of the flower, including carpel-enclosed ovules and proximity of male and female organs. The SEPALLATA (SEP1-4 in Arabidopsis thaliana) subfamily of MADS genes encode transcription factors specific to angiosperms and act as organizers of MADS tetrameric complexes required for flower and ovule identity. The MADS complexes characterized in angiosperm contain at least one SEP protein. In the absence of SEPs,

no floral organs form. However, the putative role of SEPs in ovule and seed development was less clear. Genetic evidence demonstrating the requirement of SEP genes in seed development was lacking because *Arabidopsis* single sep mutants lack floral phenotype and in sep1sep2sep3 mutants, the flower shows complete homeotic conversion of carpel to sepal with no ovule. To address the specific role of SEPs in seed development, we designed a new version of Arabidopsis sep1sep2sep3 mutant that decreases the level of SEP-related MADS complexes but still allow reproductive organ development. Nevertheless, the mutant impedes ovule and seed development, resulting in clear seed phenotypes. These defects are fully rescued with SEP3, highlighting the requirement of SEP protein in seed development. Our unpublished data will be presented and discussed in the context of the requirement of SEP protein for the successful reproduction in angiosperms.

Deciphering the diversity of the 3D cellular basis of ovule curvature

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Tissue morphogenesis remains poorly understood. In plants, a central problem is the interplay between cellular behavior of mechanically connected cells and tissue-level processes that lead to emergent properties. We use ovule curvature as a model to address this aspect, taking a comparative approach that exploits the diversity of ovule curvature across angiosperms. We apply advanced imaging and machine learning based cell segmentation to generate 3D digital ovules with single cell resolution. This allows us to investigate the hidden functional complexity of the 3D cellular architecture underlying ovule curvature. We then combine quantitative comparative morphometry and topological analysis to explore similarities and differences in the 3D cellular architectures of ovules from a variety of angiosperm species. The cellular parameters obtained are used in finite element modeling (FEM) to develop plausible models that explain the differences in ovule curvature, which in turn are functionally tested by genetics where possible. Here we present the results of our work on two species with differently shaped ovules: *Arabidopsis thaliana* and *Cardamine hirsuta*. We first generated 3D digital atlases of ovule development at single cell resolution for both species. Analysis of these 3D digital ovules combined with FEM suggests that subtle differences in cellular growth patterns in the region flanked by the integuments result in the striking differences in ovule curvature between the two species. Our work demonstrates the power of comparative 3D cellular morphometry and the importance of internal tissues and their developing cellular architecture in inducing the emergent properties characteristic of tissue morphogenesis.

Tools for genetic studies of the model fern *Ceratopteris richardii*

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Ferns and their allies (monilophytes) represent the second most species-rich group of land plants and are of considerable ecological importance. As a sister group of seed plants (including flowering plants) they are also of great evolutionary interest. Compared to flowering plants, however, much less is known about the developmental and molecular biology of ferns. MADS-box genes encode MADS-domain transcription factors that play important roles in the development and evolution of land plants. Especially well-characterized is their role as organ identity genes during

flower development of angiosperms. Even though the first MADS-box genes have been molecularly cloned from ferns almost 30 years ago, little is known about their function, mainly due to a lack of mutants. Also their quite broad expression domain, often comprising both the gametophytic and the sporophytic phase of the life cycle of ferns, provide few specific clues concerning gene function. To further facilitate studies on fern biology we aim at simplifying genome engineering of *C. richardii* with the CRISPR-Cas9 system. We report *C. richardii* plants that express Cas9 nuclease under control of the strong CaMV 35S promoter. For efficient expression of single guide RNA (sgRNA) by RNA polymerase III we identified *C. richardii* U6 promoters. We also created transgenic plants for overexpressing the MIKCC-type MADS-box gene CRM3. First results show that overexpression of CRM3 affects sporophyll development in *C. richardii*, possibly by inhibiting cell proliferation or elongation. These technical improvements may foster many fields of fern physiology, development and evolution.

Finding the 'seed' in seedless plants

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Plants have not always reproduced using seeds, which evolved in a single group of land plants, the spermatophytes (gymnosperms and angiosperms). Seed-based reproduction evolved from an older mode of plant reproduction still found in the surviving descendants of seedless plant lineages. The seed represents a novel plant organ which conferred a huge selective advantage on the spermatophytes, but the question of how plant development was reprogrammed to first form the seed has remained intractable. The closest living seedless relatives of all extant spermatophytes are the ferns, whose reproduction can be used as a proxy for the ancestral seedless mode of plant reproduction and to study the evolution of the seed at the genetic level. In ferns, seed functions are found in two separate and developmentally-distant organs: meiosis occurs in spore-bearing fronds (sporophylls) on the diploid plant, while egg generation, fertilization and embryo development occur in egg chambers (archegonia) borne on a separate, free-living haploid gametophyte. In-depth RNA-seq analysis of the development of both of these organs in the fern Ceratopteris richardii has identified significant conservation of gene networks between the Arabidopsis seed and the fern post-fertilization archegonium, but not the sporophyll. This finding raises the hypothesis that the diploid sporophyll may have been modified into the seed through mis-expressing ancestral reproductive gene networks from the haploid archegonium.

SESSION 4
Evolution of Carpels and Fruits
Chair: Annette Becker (Gießen)

Development sculpting floral sexuality and floral fertility in the grasses

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Floral development governs gamete production in angiosperms, including when and where fertile flowers are available to make seeds. In the grasses, flowers occur in

structures called spikelets. In the Panicoideae, the grass subfamily which maize and many other crops belong to, spikelets contain two flowers, but usually only one flower (the upper flower) produces a grain after pollination. This is because floral organs, particularly the carpels, are suppressed in lower flowers, leading to staminate or completely sterile flowers. Thus, the potential to produce two grains per spikelet is limited by carpel suppression. Carpel suppression, mediated by programmed cell death, likely evolved in the lineage leading to the Panicoideae, suggesting that homologous genes regulate carpel suppression across the subfamily. In this era of genome engineering, carpel suppression genes could be transformative targets for genome editing and yield improvement in panicoid crops. However, only a few genes are known to regulate carpel suppression in maize and its relatives. Furthermore, how these genes interact in pathways, and whether these genes have conserved functions in panicoid grasses remains largely unknown. Here, I will outline our work in identifying the genes that control carpel suppression in maize, and our ongoing work in assessing their function and evolution in the Panicoideae and the grass family more broadly.

The problem of carpel identity and gynoecium diversity in grasses (Poaceae)

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The carpel is the basic unit of the angiosperm gynoecium. The evolutionary origin of the angiosperm flower remains unresolved, and carpel homologies remain problematic. Despite the contrasting views proliferated over the 20th century, there is currently a widespread consensus that there was a single origin of carpels in angiosperms. Two distinct zones, the ascidiate and plicate zone, can be distinguished in many angiosperm carpels; when there is only one ovule per carpel, it is inserted ventrally at the boundary between these two zones. Of special interest are the gynoecia that resemble this archetypal groundplan but are unlikely to be composed of just a single carpel. We discuss this issue using grasses (Poaceae), an economically and ecologically important family that incorporates several model organisms. Grasses have a single ovule per ovary. In the grass gynoecia with two stigmas, these belong to two sterile carpels, and the ovule belongs to a carpel that lacks a stigma. In a few monostigmatic or almost monostigmatic grasses, the gynoecium resembles a single carpel. Depending on the species, these examples can be interpreted as (1) fusion of the stigmas in the sterile carpels, (2) regain of a stigma in the fertile carpel, with the two other stigmas being lost or reduced in size and fused to it, or possibly (3) 'simple' variation in the number of stigmas. It remains an open question whether the carpel still exists as a natural phenomenon in grasses or their ancestral carpels became completely integrated in a novel unit.

The role of *LEUNIG* and *SEUSS* transcriptional regulators during land plant evolution

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The adaptation of reproductive strategies to the terrestrial environment was one of the crucial steps of the transition from water to land that plants had to overcome. During land plant evolution, various adaptations emerged, from the simple, water-dependent

fertilization mechanism of bryophytes to the morphologically highly diverse flowers of angiosperms. This wide range of reproductive strategies makes it especially noteworthy that some of the essential regulators of flower development in angiosperms are present in all major land plant lineages. In Arabidopsis thaliana, the transcriptional regulators LEUNIG (LUG) and SEUSS (SEU) play an important role in plant and flower development. SEU can act as both a transcriptional repressor by forming a LUG-SEU heterodimer that interacts with floral organ identity proteins like APETALA1 and the histone deacetylase HDAC19, but also as a transcriptional activator, for example by forming a complex with SDG4. The fern *Ceratopteris richardii*, the moss *Physcomitrium patens* and the liverwort *Marchantia polymorpha* encode, like *Arabidopsis*, several orthologs of LUG and SEU, raising the questions of (1) how exactly did these regulators co-evolve with each other and other transcriptional regulators to become such important floral regulators, and (2) if their ancestral function was also related to sexual development. We use Yeast-Two Hybrid and Bifluorescence Complementation assays to study protein interactions between the orthologs of LUG, SEU and their most prominent interaction partners in *Arabidopsis*, the MADS-Box transcription factors. We also analyze the domains important in the co-evolution between LUG and SEU, heterodimer formation and for target protein interaction.

Characterisation of *MADS* transcription factors complexes in Gymnosperms and the origin of the flower

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The flower is a major reproductive innovation in the seed plant lineage and how floral organs are produced has been extensively studied. The identity of floral organs is determined by the combinatorial activity of the MADS transcription factors (MTFs). One hypothesis concerning the origin of the flower is the modification of protein-protein interaction patterns and duplication events within this family during plant evolution. In angiosperms, MTFs are divided into classes A to E, and all floral organ development requires MADS tetramers with at least one class E MADS. For example, tetrads composed of class C/E MADS are required for the development of carpels. However, in gymnosperms, the sister clade of angiosperms in which reproductive organs are separated into conical structures, only class B and C MADS are present, with no class E identified to date in existing species. Despite preliminary data suggesting that tetramer formation in non-flowering plants could be independent of class E, the nature of functional MTF oligomers in gymnosperms is still largely unknown. Focusing on the development of female organs, our aim is to reveal the rules governing the formation and function of MADS complexes in gymnosperms, and to decipher the importance of the appearance of E in the origin of flowers. To this end, homologous C-class proteins from four species representing each gymnosperm subclass were analysed using a multi-scale approach including structural and biochemical studies and in planta complementation assays. Recent data on oligomerisation capacity, and DNA-binding activity, and functionality will be presented.

How to grow apart: the evolution of sex determination in *Cannabis sativa*

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The vast majority of flowering plants are hermaphroditic, with male and female reproductive organs developing in the same flower. However, around 6 % of flowering plants are dioecious, i.e. develop separate male and female individuals. Dioecy originated multiple times independently during evolution, yet the molecular pathways involved in sex determination remain poorly understood. We aim to characterize the genetic mechanism controlling sex determination and sexual dimorphism in one of the world's oldest crops, *Cannabis sativa*. We show that male and female individuals diverge morphologically already at a very early developmental stage in *Cannabis*, and that profound differences exist between male and female individuals. Using genetic mapping, transcriptomics, sex chromosome analyses and morphological studies we provide evidence that a number of gene regulatory proteins encoded on the sex chromosomes contribute to sexual dimorphism in *Cannabis*. We identify a region on the X chromosome that may be a "hot spot" for the evolution of sex determination. We postulate that genes implicated in carpel and ovule development accumulated in this region over evolutionary time scales, eventually resulting in the differences between male and female flower development we see today in *Cannabis sativa*.

The mechanism and evolution of ovule curvature and its importance to hypotheses for the origin of angiosperms.

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Several extinct gymnosperm groups have been proposed as potential ancestors or close stem-relatives of angiosperms. Among these, Caytoniales contain multiovulate cupules which exhibit a shape reminiscent of that of the outer integument in angiosperm species that possess curved ovules. It has accordingly been argued that the Caytoniales cupule may be an evolutionary precursor to the angiosperm outer integument. Ovule curvature in *Arabidopsis thaliana* is known to be caused by the asymmetric expression of INNER NO OUTER (INO), encoding a YABBY transcription factor, which promotes outer integument expansion more strongly on the underside of the ovule. The asymmetry of INO expression in Arabidopsis is due to a negative genetic interaction with the C2H2 zinc-finger transcription factor SUPERMAN (SUP), which is known generally to downregulate its target genes through recruitment of the polycomb repressive complex2 (PRC2). However, the exact physical nature of the interaction between SUP and INO in *Arabidopsis* is unknown, as is the degree of conservation of this regulatory mechanism among angiosperms showing curved ovule symmetry. We show that the negative regulation of INO by SUP in *Arabidopsis* does not occur, as had been previously hypothesized, through the presence of SUP-binding sites in the INO coding sequence. Instead, this interaction appears to depend on a specific property of the *Arabidopsis* INO protein that is shared with its ortholog from the basal angiosperm Nymphaea thermarum, also

possessing curved ovules. However, INO orthologs from species showing straight ovules, including the basal angiosperms *Amborella trichopoda* and *Barclaya longifolia* (a close relative of Nymphaea), are not negatively regulated by SUP in transgenic *Arabidopsis* and accordingly promote outer integument development equally on both sides of the ovule. We conclude that the developmental decision to produce curved or straight ovules is dependent on a cryptic property of the INO protein, and that an INO-based mechanism to promote ovule curvature has been conserved from a very early stage in angiosperm evolution. We discuss these and other findings in relation to hypotheses for the origin of the angiosperms.

SESSION 5
Evolution of Fertilization Mechanisms in Plants
Chair: Stefanie Sprunck (Regensburg)

Evolutionary insights into double fertilization

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Double fertilization is a unique mechanism in flowering plants, playing a critical role in their evolutionary success. Compared with the pollen tubes of gymnosperms such as cycads, which produce motile sperm, potential sensory receptor kinases have undergone significant evolutionary changes (Toyama et al., in preparation). Rapid tip growth and guidance in flowering plant pollen tubes are achieved through complex pollen-pistil interactions (e.g., Mizuta et al., 2024, EMBO Rep.). In addition to directional and growth cues provided by peptides, the regulation of capacitation in the rapid fertilization process is unique to flowering plants. Non-proteinaceous cues, such as steroid hormones and sugar chains, also play important roles (Matsuura-Tokita et al., submitted; Mizukami et al., 2024, RSC Chem. Biol.; Kato et al., in preparation). True gamete fusion, involving two male and two female gametes, occurs deep inside the pistil, where unknown mechanisms strictly determine fertilization targets. In this talk, I will present evolutionary insights into double fertilization in flowering plants, focusing on some of these molecular factors.

Evolution and function of *EC1* and other *ECA1* gametogenesis-related small secreted cysteine-rich proteins

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Small secreted cysteine-rich proteins (CRPs) are found in all higher eukaryotic organisms. Their functions are diverse and range from antimicrobial peptides to extracellular enzyme inhibitors to ligands for plasma membrane-bound receptor kinases and more. In the plant kingdom, angiosperms stand out with their abundance of functionally diverse CRPs. The family of “Early Culture Abundant 1 (ECA1) gametogenesis-related” CRPs is found in all angiosperms, including *Arabidopsis thaliana* and the basal angiosperm *Amborella trichopoda*, and has been described as angiosperm-specific. Despite the large abundance of at least 124 ECA1

gametogenesis-related CRPs in *A. thaliana*, only the function of the small subfamily of EGG CELL1 (EC1) proteins has been described so far. The *Arabidopsis* EC1 family consist of five functionally redundant proteins that are important for sperm cell activation and subsequent fusion of the two gamete pairs during double fertilization. When the sperm cells are released from the pollen tube, EC1 proteins are secreted from the egg cell. This triggers the translocation of the gamete fusogen HAP2 and the fusion facilitator DMP9 to shift from the endomembrane system to the sperm cell plasma membrane, allowing the gametes to fuse. A Cas9-free fivefold knockout mutant of the EC1 genes (5xec1) leads to significant defects in double fertilization, with sperm-egg fusion being more severely affected. As a result of gamete fusion failure, we observe a drastically reduced seed set. In this project, we investigate the evolutionary history of ECA1 gametogenesis-related CRPs in land plants and their tissue-specific expression patterns. We also aim to elucidate the relationship between the structure and function of EC1 and other members of the ECA1 gametogenesis-related CRP family. Here we report the results of our search for EC1 and other ECA1 gametogenesis-related CRPs in different land plant species, our homology-modelling of the protein structures, and the identification of functionally relevant domains in EC1 and EC1-related proteins by determining their ability to restore the reproductive phenotype in the 5xec1 mutant.

Science to soil: Unlocking three-parent breeding for sustainable agriculture

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Polyploidy, a key driver of plant evolution and diversity, has traditionally been attributed to meiotic aberrations that deliver unreduced chromosome sets. Our research, however, identified an alternative pathway to polyploidization: polyspermy. We have demonstrated that plant egg cells can fuse with two sperm cells, resulting in offspring with three parents—one mother and two fathers. Using a novel polyspermy detection assay, we further showed that this innovative approach enables selective polyploidization of the egg cell, bypassing the hybridization barrier imposed by the endosperm. In addition, we have identified the first molecular factors ECS1 and ECS2, that regulate the frequency of polyspermy and three-parent plant formation in plants. Here, we discuss our result addressing, in addition, genetic and external factors affecting polyspermy and the occurrence of triparental plants. Recognizing the evolutionary and breeding significance of three-parent plants, we have developed a multiparametric high-throughput detection pipeline to identify triparental plants, unlocking new opportunities to create novel crops for a sustainable future. Acknowledgments: We acknowledge support by European Research Council (ERC Proof of Concept grant ‘TriVolve’ project ID. 957547 and ERC Consolidator Grant 'bi-BLOCK' ID. 646644) and the European Innovation Council (EIC Transition '3P-Tec' 101057189) to Rita Groß-Hardt.

Tissue-specific transcriptome analysis and chemotactic behavior observation reveal the intermediate evolutional state in male reproductive cells of gymnosperm cycad

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Cycads and ginkgo combine ancestral and derived features in the evolution of fertilization mechanisms from zooidogamy to siphonogamy in land plants. Male reproductive cells in these plants possess pollen tubes like seed plants and flagellated sperm like basal land plants. While their morphological traits are well-observed, molecular and physiological properties remain unclear. This study analyzed the physiology and gene expression of *Cycas revoluta* sperm and pollen tubes. A semi-in vivo sperm attraction assay revealed that sperm was likely attracted to chemical signals derived from female gametophyte tissues, like motile sperm in other land plants. Tissue-specific transcriptome analyses, including male reproductive cells and other cycad tissues, revealed a reduced ratio of genes associated with transcription, translation, and related processes a trend consistent across land plants. This indicates a shared expression control mechanism accompanied by the replacement of somatic histones with male-specific variants. Additionally, sperm expressed both cell membrane-localized factors found in motile sperm of basal land plants and those commonly observed in seed plant sperm cells. This suggests that cycad sperm are in a transitional state from motile sperm to immotile sperm cells and that their interaction mechanism with female reproductive tissues is also in a transitional state. Instead, pollen tube-specific receptor genes reported in angiosperms were not identified. Cycad pollen tubes probably only retain haustorial functions. These findings suggest that the loss of sperm motility might precede the evolution of functional pollen tubes like those in angiosperms, providing insights into the dynamic evolution of fertilization mechanisms in land plants.

Accessing the inaccessible: Microinjection in *Arabidopsis thaliana* reproductive cells

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Reproduction drives all living organisms. In flowering plants, the female gametophyte houses the female gametes and is the site of fertilization and seed formation. However, its deep embedding in maternal tissue makes it difficult to access and manipulate without causing significant damage. Microinjection has long been established as a method for delivering substances to single cells, and we previously developed the first protocol for microinjecting the *Arabidopsis thaliana* central cell. Here, we introduce protocols to microinject egg cells, zygotes, and germinated pollen grains using an adapted micromanipulation setup, an optimized workflow, cell type–specific markers, and an in vitro tissue cultivation system to facilitate post-injection analyses over time. We demonstrate targeted gene silencing of egg cells, embryo formation from injected zygotes, and robust microinjection of germinated pollen grains (including intracellular mitochondrial labeling). Finally, we provide evidence that synergid cells are highly susceptible to mechanical stress, supporting the hypothesis that pollen tube penetration during fertilization contributes to their disintegration.

The evolution and roles of endopeptidases in plant fertilization

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The aspartic endopeptidases play a critical role in plant fertilization. We found two aspartic endopeptidases, ECS1 and ECS2, exclusively expressed in *Arabidopsis* egg cell. Both aspartic endopeptidases could be secreted to the extracellular space from a cortical network located at the apical domain of the egg cell after successful fertilization. ECS1 and ESC2 specifically cleave the pollen tube attractor LURE1 to prevent polytubey, demonstrating that plant egg cells could sense successful fertilization and elucidate a mechanism established by fertilization-induced degradation of attraction factors to block polytubey. Further investigation reveals that the two aspartic endopeptidases also function to directly block polyspermy. We also reveal that the molecular machinery of the polyspermy block evolved in three steps originating from common ancestors of land plants. This co-evolved molecular module represents a quick and direct strategy to prevent polyspermy and ensure double fertilization.

SESSION 6
From ROS to hormones: Regulatory processes shaping land plant growth and fertility
Chair: Stefanie Müller-Schüssele (Kaiserslautern)

Phenolic oxidative polymerization as an unexplored facet of cuticle formation

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Plant cuticles are one of the most important evolutionary innovations that facilitated plant transition from aquatic to terrestrial environments. They are often regarded as impermeable cell wall modifications, representing effective barriers that protect plants from environmental stresses, while also functioning as insoluble materials that enable proper separation of organs during development. Plant cuticles comprise heterogeneous matrices combining lipids, polysaccharides, and phenolics in a complex fashion. Although phenolics are relatively minor components in the cuticles of aerial organs in vascular plants, recent data from moss gametophore (bryophyte) suggest they may play a crucial role in maintaining the structural integrity of the cuticle. Our research further points to the possibility that phenolics are incorporated into the cuticle via oxidative polymerization, in a process analogous to that occurring during lignification, defining an explored facet of cuticle origins.

Interplay of the *TGA* transcription factor *MpTGA* and its glutaredoxin co-regulators the *ROXYs* in *Marchantia polymorpha* sexual reproduction

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During the terrestrialization of plants around 470 million years ago, significant developmental and metabolic innovations emerged. While almost all transcription

factor families were already established, a novel glutaredoxin (GRX) group, the CC-type GRXs also called ROXYs arose in the common ancestor of land plants. ROXYs were shown to act in angiosperms in post-translational redox-modification of TGA TFs and can thereby modulate their activities. In *Arabidopsis*, the 10 TGA transcription factors regulate diverse developmental and stress-related response. In *Marchantia polymorpha* only one TGA transcription factor exist, MpTGA. Recently, we showed that MpTGA is a key developmental regulator, controlling the switch from vegetative to reproductive development in *M. polymorpha*. Additionally, MpTGA also affects the formation of oil bodies, which are liverwort-specific organelles involved in herbivore defense. The number of ROXY genes is significantly lower in *Marchantia*, which has only two ROXY genes (MpROXY1 and MpROXY2), in contrast to 21 ROXYs genes found in *Arabidopsis thaliana*. Notably, MpROXY1/2 can complement the roxy1 *Arabidopsis* flower phenotype, demonstrating highly conserved biochemical capacities of ROXYs during land plant evolution. MpTGA proteins interact in a redox-sensitive manner with MpROXY1/2 and their common DNA-binding depends on the cysteine redox status of MpTGA and MpROXY proteins. Here, we will present MpROXY1/2 mutant analyses and possible activities in *Marchantia* sexual reproduction will be discussed.

Conservation of sporophyte secondary cell wall development and pectin biosynthesis in land plants by Class II *KNOX*

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Land plants evolved from an ancestral alga around 470 million years ago, evolving complex multicellularity in both haploid gametophyte and diploid sporophyte generations. The evolution of water conducting tissues in the sporophyte generation was crucial for the success of land plants, paving the way for the colonisation of a variety of terrestrial habitats. Class II KNOX (KNOX2) genes are major regulators of secondary cell wall formation and seed mucilage (pectin) deposition in flowering plants. Here we show that in the liverwort *Marchantia polymorpha* loss-of-function alleles of the KNOX2 ortholog, MpKNOX2, or its dimerization partner MpBELL1, have defects in capsule wall secondary cell wall and spore pectin biosynthesis. Both genes are expressed in the gametophytic calyptra surrounding the sporophyte and exert maternal effects, suggesting intergenerational regulation from the maternal gametophyte to the sporophytic embryo. These findings also suggest the presence of a secondary wall genetic program in the non-vascular liverwort capsule wall with attributes of secondary walls in vascular tissues.

Maternal regulation of nutrients availability plays a pivotal role during ovule and seed development

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In angiosperms' ovules, fertilization of both the egg and the central cell is required to ensure proper seed development. ARABIDOPSIS Bsister (ABS) and SEEDSTICK (STK) encode for MADS-domain transcription factors (TFs), which are expressed in

the ovule integuments and seed coat, that are required for proper ovule differentiation and seed development. In the abs stk double mutant, the seed set is largely affected resulting in a few viable seeds. Moreover, the double mutant ovules show defects in integuments differentiation, together with a large amount of starch inside the embryo sac. Although most of the abs stk ovules are properly fertilized, as demonstrated by the presence of the zygotes, the development of the embryo is drastically delayed and arrested a few days after fertilization in most of the developing seeds. To unveil the roles of ABS and STK, during ovule and early seed development, we studied the abs stk double mutant phenotype by employing ChIP-Seqencing, transcriptomic and metabolomic approaches. This broad analysis has shown that sugar metabolism is widely targeted by these two TFs and impacted in the double mutant. Additionally, by employing two different approaches, we were able to modulate the starch accumulation in the abs stk double mutant, partially rescuing the embryo and seed developmental defects. Finally, by checking callose deposition we postulate a model that involves proper modulation of nutrient flow between the ovule to seed transition.
Concluding, these data highlight the importance of the interplay between metabolic and developmental processes regulated by the maternal tissues on the products of fertilization.

Pollen-mediated control of ovule development in *Ginkgo biloba*: Insights into auxin regulation and epigenetic mechanisms

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In angiosperms, pollination and fertilization are close in time. In gymnosperms, such as *Ginkgo biloba*, pollination precedes fertilization by 4/5 months. Notably, pollen capture is crucial in the differentiation of the female gametophyte and the seed coat. Specifically, our previous study demonstrated that pollen capture affects auxin distribution, represses the expression of genes involved in programmed cell death, and activates genes associated with cell cycle regulation and DNA replication, whereas the opposite occurs in unpollinated ovules. The aim of the present study was to elucidate the mechanism by which pollen controls ovule development. Consequently, ovules were manually pollinated with non-viable pollen or treated with exogenous auxin and monitored over subsequent days and analyzed for growth kinetics, histology, and gene expression. Our results suggest that, unlike auxin treatment, the mere arrival of pollen is initially sufficient to induce an increase in ovule size. However, approximately ten days later, ovules begin to abort, indicating that pollen germination, which typically occurs about one week post-pollination, serves as a secondary checkpoint. Furthermore, ChIP-seq experiments performed on pollinated ovules before and after germination revealed changes in the distribution of H3K27me3-marked regions across the genome. These findings suggest that pollen arrival reduces the activity of the Polycomb Repressive Complex 2 (PRC2), targeting genes involved in auxin biosynthesis, whose epigenetic regulation after fertilization has already been documented in *Arabidopsis*. Overall, our results indicate the presence of a conserved regulatory mechanism and provide new insights into the molecular network governing ovule development in non-model plants.

Evolution of receptor kinases signaling in land plants: from immunity to reproduction

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Receptor kinases (RKs) govern fundamental aspects in plants, from reproduction to immunity. Phylogenetic analyses revealed that RKs families underwent an expansion in land plants. Notably, some RKs such as the leucine-rich repeat (LRR) RK excess microsporocytes 1 (EMS1) or the Catharantus roseus receptor-like kinase 1-like (CrRLK1L) FERONIA have orthologous sequences in all land plant genomes. EMS1 and FER regulate the development and fertility of the male and female gametophyte in *Arabidopsis*, respectively. *Marchantia polymorpha* Mpcrrlk1l mutants show gametophytic developmental defects including rhizoid burst. We found that in addition to its function in development, MpCrRLK1L is a suppressor of plant immunity. We observed that MpEMS1 is also involved in gametophyte development in *Marchantia* and activates the panbrassinosteroid signaling pathway. Using a comparative evolutionary approach we are currently characterising upstream and downstream signaling components to assess the functional conservation of RKs signaling pathways in land plants.

SESSION 7
Co-Evolution of Signaling Systems and Gene Regulatory Networks in Plant Development
Chair: Sabine Zachgo (Osnabrück)

Reproductive strategy control in *Marchantia polymorpha*

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By controlling their reproductive strategies, plants strike a balance between generating genetic diversity or maintaining stability, promoting long-term survival in a range of environmental conditions. The liverwort *Marchantia polymorpha* propagates clonally through vegetative gemmae or via sexually generated spores, but our understanding of the genetic pathways underpinning reproductive decision-making is limited. GRAS transcriptional regulators are highly conserved proteins across land plants, with diverse functionality in stress and development. Here, we discover a central role for the SCLA-type transcription regulator MpGRAS7 in both stress signalling and reproduction in *Marchantia*. MpGRAS7 is highly expressed in gemma cups and gametangiophores and is activated in response to Far Red (FR) light and abscisic acid (ABA) environmental cues. Genetic dissection further suggests MpGRAS7 is required to maintain gemma dormancy, gemma cup frequency, and FR-induced gametangiophore development. The sequence conservation and expression patterns of MpGRAS7 orthologs in flowering plants hints towards the broader function of SCLA-type GRAS proteins in stress-regulated developmental transitions in plants.

Contribution of the *TGA* TF to the evolution of sexual reproduction

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TGACG-binding (TGA) transcription factors (TF) are known in angiosperms as pivotal regulators of developmental processes and stress responses, acting through interactions with co-factors. In the flowering plant *Arabidopsis thaliana*, TGA TFs interact with various co-factors, including members of the NON-EXPRESSOR OF PATHOGENESIS-RELATED (NPR) protein family, to mediate functions such as plant growth and immunity. However, the role of TGA and NPR proteins in non-flowering plants, such as mosses, liverworts and hornworts, is still largely unexplored. Our recent studies on the liverwort *Marchantia polymorpha* revealed that its single MpTGA exhibits two main functions: it induces together with MpNPR the formation of stalked sexual structures, the gametangiophores, and also independently regulates oil body formation. These two MpTGA functions control the formation of synapomorphic structures that are unique characteristics of complex thalloid liverworts, prompting intriguing questions about the diversification and functional roles of TGAs in other bryophyte lineages. To address this question, we investigate representative species from distinct bryophyte lineages. The hornwort *Anthoceros agrestis* possesses one TGA protein but has lost the NPR protein, whereas the moss Physcomitrium patens exhibits an expanded gene repertoire, with four TGA and two NPR proteins, suggesting lineage-specific gene duplication and functional diversification. Additionally, the amphibious liverwort Riccia fluitans is another interesting liverwort as it likely lost the distinctive TGA-regulated synapomorphies found in complex thalloid liverworts and contains, like *Marchantia*, a single TGA und NPR protein. Here, we present preliminary findings on the diversification of TGA functions in these bryophytes.

Conservation and divergence of the *FERONIA* signal transduction pathway in *Marchantia polymorpha*

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Research in our laboratory focuses on the developmental genetics of plant reproduction, with an emphasis on cell-cell communication during fertilization. In the flowering plant *Arabidopsis thaliana*, fertilization depends on the proper reception of the pollen tube by the synergid cells, involving a complex signaling interplay mediated by receptor kinases of the CrRLK1L subfamily. Since its discovery as a key player in plant reproduction, the FERONIA (FER) receptor kinase has been shown to play a role in a multitude of physiological and developmental processes, including cell expansion, hormone signaling, and innate immunity. Over the last decade, the extracellular domain of CrRLK1Ls has been reported to bind - together with its coreceptors of the LORELEI (LRE) family - ligands of the RAPID ALKALINIZATION FACTOR (RALF) family, as well as pectin and peptides of the PCP-B family. Studying the diverse functions of CrRLK1L signaling in *Arabidopsis* is complex because of high redundancy of its core components: with 17 CrRLK1Ls, 37 RALFs, and 4 LREs, a theoretical total of 2516 combination for the ternary

receptor complex are possible. Therefore, we have turned to the evolutionary distant model *Marchantia polymorpha*. With only a single CrRLK1L (MpFER), two LREs (MpLRE1/2), and three RALFs (MpRALF1/2/3) encoded in the *Marchantia* genome, it should be much easier to decipher the key roles and mechanisms underlying this important, plant-specific signaling pathway. We will report on our recent advances in characterizing the components of the FER signaling pathway in *Marchantia* and its conserved and divergent functions, respectively.

Arrested development: How do plants control the timing of identity transitions in embryonic stomatal cells?

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During plant development, cell patterning is tightly regulated to ensure each cell acquires a suitable cell fate. While extensive studies have revealed factors that control spatial regulation of cell fate, less is known about regulation of developmental timing: what factors control when a cell can move to the next cell state in its lineage? In our new lab we are using identified embryonic developmental blocks/delays in the stomatal lineage to identify mechanisms that control developmental timing. Stomatal patterning should be both flexible and robust: adjusting to the plant's environment while maintaining spacing. Across eudicot species, I found that embryonic stomatal pre-patterning occurs during the final stages of embryogenesis, with species that experience early desiccation lacking embryonic pre-pattern. The delay of stomatal patterning until the final embryonic stages is surprising considering the early expression of the stomatal regulator SPEECHLESS (SPCH). SPCH is expressed in developing cotyledons from heart stage onwards. I had initially found that increasing SPCH levels during embryogenesis was not sufficient to induce early stomatal patterning but now see that activity of SPCH's partner, SCREAM (SCRM) might play a role in this delay. In addition, we've started testing the hypothesis that during embryogenesis, regulation of stomatal patterning might resemble the stem instead of the leaf, with TOO MANY MOUTHs having a positive instead of its negative effect on the formation of stomatal precursors. Altogether, we are identifying components regulating the embryonic stomatal pattern and determining how wiring of this network is similar to or different from post-embryonic development.

Mechanical control of germ cell specification in Arabidopsis anthers

Chan Liu, Hui Shi, Yuting Han, Pan Wang, Kexin Li, Zhishuai Zhang, Jiazheng Liu, Yafeng Zheng, Linlin Li, Limei Lin, Chen Liang, Binjun Qin, Hua Han, Shunong Bai, Xiao Liu, Wenqian Chen, Feng Zhao
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A central question in developmental biology is how the germline is established. We have studied the specification of the male germ cells (GCs) within the anther. Hereby, we have focused on the potential role of mechanics, an aspect of anther development which has been very poorly characterized. Using a combination of live imaging and mechanical measurements, we provide evidence that GCs originate in a special micro-mechanical niche, where inner tissues exert 'push' on outer cell layers, placing them under compression. Mechanical perturbations significantly disrupted the GC specification and patterning. Moreover, we found that the master genetic regulator SPOROCYTELESS/NOZZLE (SPL/NZZ) is central in establishing this micro-

mechanical environment by softening the cell wall. The mechanical cues, in turn, stabilize the transcription of SPL/NZZ. We propose here an intrinsic growth-derived mechano-chemical feedback loop that drives germ-cell fate acquisition.

Unraveling Epidermal Cell Differentiation in Arabidopsis Flowers

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Epidermal cell morphology varies widely across floral organs, with specialized cell types such as petal conical cells, stigma epidermis, giant sepal cells, anther stomium, and nectary secreting cells. In this study, we analyzed cellular differentiation trajectories in developing *Arabidopsis* flowers and identified a key role for MYB transcription factors in specifying epidermal cell identities downstream of homeotic regulators. Our findings provide new insights into the genetic control of epidermal specialization, paving the way for comparative studies across species to explore the molecular basis of epidermal morphology evolution in flowers.

SESSION 8
Bioinformatics solutions to harness the EvoDevo Data Universe Chaos
Chair: Alexander Goesmann (Gießen)

Cross-plant comparison of gene expression

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An important question in the study of plant development is understanding the acquisition of new traits in the course of evolution as plants adapt to new environments. As an example, land plants have evolved flowers to improve reproduction. One driving force for the development of such new traits is the change in the function of genes. Gene expression studies can help to determine in which tissue types genes are expressed, and new functions of genes can be discovered by comparing the expression profile of orthologous genes from different plant species across multiple tissues. Here we present methods and databases to analyze expression data from plants to help researchers compare the expression of genes or co-expression networks across multiple plant species.

California poppy (*Eschscholzia californica*) genome and transcriptome atlas shed light on the concerted evolution of complete metabolic pathways

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The bright orange petals of California poppy (Calpop) blanket whole landscapes – not only at the US Pacific coast where it naturally occurs but also all over the Mediterranean climate zones of the Southern hemisphere where it is highly invasive. Calpop's reproductive success is partially due to carotenoid pigments attracting pollinators and the benzylisoquinoline alkaloid (BIA) biosynthesis that yields herbivore-deterring metabolites protecting also reproductive organs. This species is an

important model organism for plant evodevo and alkaloid biosynthesis and regulation. However, the molecular base of its life history traits and their evolution remains unknown. Here we present and analyze the haplotype-resolved chromosome-level reference genome in combination with a high resolution transcriptome atlas of California poppy. Our work shows that the different gene families contributing to alkaloid biosynthesis all show large phylogenetic gene clusters where similar expression pattern often coincides with high sequence similarity and close vicinity in the genome. In contrast, carotenoid biosynthesis genes mostly come in single copies per gene family but their expression pattern changed substantially in evolution and they do not form clusters. Floral organ identity genes represent a third type of evolutionary characteristics: the do not form clusters, maintain their expression pattern during evolution and show moderate increase in gene number per subfamily. Our analysis show that reproductive success of Calpop depends on the combination of evolutionary modes demonstrating that different gene families involved in similar metabolic or developmental processes preferentially evolve in a similar way.

Open Flower: A blooming model for teaching, outreach and research (& Poster n°22)

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Arabidopsis thaliana is the most extensively researched plant species, with an estimated 50,000–100,000 papers published, and substantial funding invested in its study over the past 3-4 decades. *Arabidopsis* has played a critical role in plant science and majorly contributed to fundamental insights into molecular mechanisms of plant biology. However, the direct translation of *Arabidopsis* research to agricultural or public applications has been limited due to its evolutionary distance from major crop species, public resistance to genetically modified crops, and its relatively unremarkable appearance. To address these challenges, Open Flower, an initiative aimed at establishing a collaborative framework for the domestication of *Arabidopsis* ornamental flower varieties, will be presented with three objectives: (1) to enhance public engagement with plant science, (2) to provide a unified platform for applying plant biotechnologies and directed plant evolution on a short timescale, and (3) to serve as living models for teaching. This presentation will highlight initial findings on launching all three objectives. First, advancements in modifying Arabidopsis flowers to assess their effectiveness as a public-facing promotion of education surrounding plant biotechnology will be discussed. Secondly, how applied research on Arabidopsis can provide transformative insights into plant domestication and unexplored uses of biotechnologies through leveraging its vast benefits as a model organism will be explored. Finally, strategies for integrating Open Flower into educational settings and providing the flowers to schools and universities as tools for education and research will be outlined.

Genomic imprinting and regulatory networks in seed initiation of an early-diverging angiosperm

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Genomic imprinting plays a crucial role in seed development by regulating parent-of-

origin gene expression, particularly in the endosperm. While extensively studied in some flowering plants, its evolutionary origins and functional significance in early-diverging lineages remain largely unexplored. Here, we investigate imprinting and regulatory networks governing seed initiation in Nymphaea, an early-diverging angiosperm with a distinctive reproductive strategy. By integrating single-cell transcriptomics with laser-capture microdissection and bulk transcriptome profiling, we identify distinct regulatory modules controlling seed compartment differentiation. Our analysis reveals a conserved core of imprinted genes, as well as lineage-specific imprinting patterns associated with hormonal signaling, chromatin remodeling, and transcriptional repression. Notably, we uncover a network of imprinted transcription factors and epigenetic regulators that likely mediate seed initiation and early growth. These findings suggest that while imprinting mechanisms in Nymphaea share commonalities with other angiosperms, they also exhibit unique regulatory innovations. Our study provides a comparative framework for understanding how imprinting and gene regulatory networks have shaped seed evolution across flowering plants, offering insights into the early diversification of reproductive strategies.

Genomic and phenotypic coevolution in land plants

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Plant evolution has been characterised by a series of major novelties in their vegetative and reproductive traits that have led to greater complexity. Underpinning this diversification has been the evolution of the genome. When viewed at the scale of the plant kingdom, plant genome evolution has been punctuated by conspicuous instances of gene and whole-genome duplication, horizontal gene transfer and extensive gene loss. The periods of dynamic genome evolution often coincide with the evolution of key traits, particular reproductive innovations, demonstrating the coevolution of plant genomes and phenotypes at a macroevolutionary scale. I will discuss how phylogenomics has furthered our understanding of plant evolution, from the earliest land plants through to extant diversity.

Poster Abstracts

FLASH TALKS & POSTERS

Role of DNA methylation in asexual endosperm formation (Poster n°1)

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Most angiosperms produce seeds in a fertilisation dependent manner. Apomictic species, however, can produce clonal offspring genetically identical to the maternal parent. This is a highly desirable trait in agriculture, as it can allow maintaining hybrid vigour and other favourable traits, and enable seed production even when

pollen/pollinators are limited. Introducing apomixis in sexual species has seen major advances recently but engineering of an autonomous endosperm, one of the key steps involved, is yet to be achieved. The manipulation of auxin levels or removal of the epigenetic repressor Polycomb Repressive Complex 2 (PRC2) can initiate endosperm development even without fertilisation, but these endosperms do not proliferate as much as sexual endosperms and they fail to cellularise, eventually being non-viable. It has been previously observed that DNA hypomethylation in a PRC2 mutant background can overcome this limitation and lead to proliferative and cellularised endosperms. However, the underlying molecular mechanisms remain unexplored. Therefore, resorting to mutants impaired in DNA methylation and to chemical inhibitors of this epigenetic mark, we wish to investigate this further and understand how DNA methylation regulates asexual endosperm development.

Duplication of MADS-box genes in land plants empowered the functional divergence between sporophytes, gametophytes and endosperm (Poster n°2)

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MADS-box transcription factors (TFs) are key regulators of reproductive development in flowering plants. Among these, MIKCC-type MADS-box TFs have well-established functions in floral development, whereas MIKC*-type MADS-box TFs primarily function in pollen. Recently, we demonstrated that two angiosperm-specific subtypes of M-type MADS-box TFs, designated as Mγ and Mα*, have evolved in concert to regulate developmental programs in the embryo sac and endosperm. Additionally, our findings revealed that all three types of MADS-box TFs are plant-specific duplicates, orthologous to animal MEF2 genes. Phylogenetic analysis indicated that the MIKCC-type clade diverged first, with the MIKC*-type clade being more closely related to the M-type clade. Based on these insights, we propose a refined model for the functional divergence of plant MADS-box TFs. In this model, the updated Type I clade—comprising M-type and MIKC*-type genes—retained ancestral functions in gametophytes while neofunctionalizing to contribute to endosperm development. In parallel, the refined Type II clade, consisting of MIKCC-type genes, evolved novel roles critical for the successful development and adaptation of sporophytes.

Evolution and development of ovule packaging variation in the *Phlox* genus (Poster n°3)

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Seed size and number are fundamental measures of maternal reproductive fitness in plants. While total seed number is dependent on many factors, ovule number per flower provides the starting point for expected seed yield per fruit. Numerous studies have highlighted the diversity of ovule number and position across major flowering plant lineages, but variation in ovule packaging is rarely observed within or between closely related species, limiting our understanding of the fundamental mechanisms governing ovule number diversity. Most species of *Phlox* produce exactly one ovule per chamber in a three-chambered ovary, however, there have been several independent transitions to packaging multiple, smaller ovules per locule. First, I compare ovary development during ovule initiation within and between six species of

Phlox (and one outgroup species), representing the ancestral single-ovulate morphology and several multi-ovulate forms. We find that, along with increasing ovary size, reduced ovule primordium diameter and a unique proliferation of the placental tissue can explain the transition to a multi-ovulate phenotype, including in a species (*P. longifolia*) that we discovered to be polymorphic for the ancestral and derived ovule packaging morphologies. Second, I discuss the polymorphism observed in *P. longifolia*, in particular the roles of plasticity and genetic variation in producing the patterns we observe across developmental time scales and between populations. Collectively, these studies will inform our understanding of how ovule packaging variation arises and ultimately results in seed size and number variation in the *Phlox* genus.

Genetic determinants of autonomous endosperm formation in *Arabidopsis* (Poster n°4)

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In Angiosperms, seed development starts with the double fertilization of the maternal gametes by two sperm cells. This leads to the formation of two distinct structures, the embryo and the endosperm, surrounded by a maternal seed coat. Some species called apomicts can produce clonal seeds without fertilization. However, this highly desirable agronomical trait is not present in crops. The apomixis process involves three steps: bypassing meiosis, embryo parthenogenesis and autonomous endosperm formation. This project aims at deciphering the molecular mechanisms involved in autonomous endosperm formation. Hormonal treatments with auxin or mutations in components of the Polycomb Repressive Complex 2 (PRC2) induce the first step of autonomous endosperm formation, but its growth and cellularization are still a limiting factor. Among PRC2 mutants, fie leads to the formation of big autonomous seeds, whereas fis2 produces small autonomous seeds. A genetic suppressor screen led to the identification of ARID2, an AT-rich interaction domain containing protein, as a genetic determinant of large autonomous endosperm in fie mutants. This protein has been shown to take part in multiple protein complexes named PEAT. Further functional studies conducted on ARID2 and different members of these PEAT complexes revealed a possible role for this complex in the genetic repression of seed development prior to fertilization in addition or in parallel with that of PRC2. These results shed light on the repression of autonomous seed development in non-apomict species and provide new keys for the engineering of autonomous seeds in crops.

Maternal control of RNA decay safeguards embryo development (Poster n°5)

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In angiosperms, the seed consists of three genetically distinct components: the embryo, the endosperm, and the maternal integuments. Double fertilization results in the formation of a diploid embryo and a triploid endosperm, both of which develop within maternal sporophytic integuments. Although a direct influence of the maternal sporophytic tissues on embryo development has been proposed, supporting molecular evidence remains scarce. Here, we show that secondary siRNAs generated by the impairment of the exosome complex cause embryo abortion through a maternal

sporophytic effect. Mutations in the exosome subunits cause embryo arrest associated with ectopic post-transcriptional gene silencing (PTGS). Complementation of the exosome using seed coat promoter-specific expression rescues this defect, demonstrating maternal control of embryogenesis via an RNA decay safety pathway. Our results suggest that siRNA overloading of AGO1 disrupts miRNA function, leading to embryo arrest. This study provides direct evidence for maternal regulation of embryonic development likely mediated by small RNA transfer from the integuments with unchecked RNA decay.

***CoExPlore*: Interactive comparative analysis of co-expression networkss (Poster n°6)**

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Co-expression network analysis is a powerful approach for identifying gene modules and characterising their relationships with biological traits. However, most available tools focus on single datasets, limiting the ability to explore common or divergent transcriptional patterns across multiple traits and species. Here we present CoExPlore, a tool for comparative co-expression network analysis. By aligning co-expression modules and their eigengenes across different datasets, CoExPlore enables users to uncover co-regulated gene groups, identify trait-related modules, and use metadata - such as GO annotations and orthogroups - to explore subsets of genes, generating refined modules and new eigengenes for deeper insights.

POSTERS

Mechanical control of germ cell specification in *Arabidopsis* anthers (Poster n°7)

Chan Liu, Hui Shi, Yuting Han, Pan Wang, Kexin Li, Zhishuai Zhang, Jiazheng Liu, Yafeng Zheng, Linlin Li, Limei Lin, Chen Liang, Binjun Qin, Hua Han, Shunong Bai, Xiao Liu, Wenqian Chen, Feng Zhao
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A central question in developmental biology is how the germline is established. We have studied the specification of the male germ cells (GCs) within the anther. Hereby, we have focused on the potential role of mechanics, an aspect of anther development which has been very poorly characterized. Using a combination of live imaging and mechanical measurements, we provide evidence that GCs originate in a special micro-mechanical niche, where inner tissues exert ‘push’ on outer cell layers, placing them under compression. Mechanical perturbations significantly disrupted the GC specification and patterning. Moreover, we found that the master genetic regulator SPOROCYTELESS/NOZZLE (SPL/NZZ) is central in establishing this micro-mechanical environment by softening the cell wall. The mechanical cues, in turn, stabilize the transcription of SPL/NZZ. We propose here an intrinsic growth-derived mechano-chemical feedback loop that drives germ-cell fate acquisition.

Dissecting transcriptional regulation of endosperm cellularization at the single nucleus level (Poster n°8)

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The endosperm, besides serving as a nutritious tissue within seeds, plays a crucial role in regulating embryo development. The precise timing of endosperm cellularization is essential for embryo survival. Despite its importance, the spatial and temporal regulation of endosperm cellularization remains poorly understood. Here, we conducted single-nucleus RNA sequencing (snRNA-seq) of endosperm nuclei before and after cellularization. Our analysis identified distinct clusters of nuclei corresponding to different endosperm domains, revealing the inherent heterogeneity of this tissue. By comparing transcriptomic changes in each cluster before and after cellularization, we identified domain-specific activity of transcription factors governing endosperm cellularization. This comprehensive approach provides novel insights into the regulatory mechanisms underlying endosperm cellularization, shedding light on its spatial and temporal control and uncovering potential targets for further investigation.

Identifying the origin of Pol4-dependent small RNAs in the endosperm (Poster n°9)

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In flowering plants, postzygotic hybridization barriers frequently arise in the endosperm, for reasons that remain to be elucidated. The inbreeder *Capsella rubella* (Cr) and the outbreeder *Capsella grandiflora* (Cg) are diploid sister species that diverged about 100,000 years ago. Despite their recent divergence, hybridization between Cr maternal plants and Cg pollen donors (Cr x Cg) causes failure of endosperm cellularization, a critical process of endosperm development, leading to embryo arrest and seed abortion. The hybrid endosperm shows chromatin decondensation and DNA hypomethylation, connected with deregulation of many genes. Similar phenotypes are also observed in seeds lacking maternal RNA polymerase IV (Pol4) activity. Pol4 primarily generates 24 nt small RNA (sRNAs) from sRNA loci that feed into the RNA-directed DNA methylation pathway, regulating activity of transposable elements as well as gene expression. Most loci that become depleted of sRNAs in the Cr x Cg cross depend on maternal Pol4 function, suggesting that loss of maternal Pol4-dependent sRNAs underlines these hybridization phenotypes. However, the origin of these sRNAs remains elusive, as well as the mechanisms by which these sRNAs affect gene expression. As sRNAs are potentially mobile, ovule or seed coat-derived sRNA may move into the endosperm to control its development. We have isolated and compared sRNA populations from the ovule, the seed coat and the endosperm. In addition, we have expressed Pol4 specifically in sporophytic and gametophytic tissues to identify the origin of the sRNA populations in the endosperm. Based on our findings, we speculate that Pol4 indeed produces sRNAs in maternal sporophytic tissues, which subsequently migrate to the endosperm, where they regulate gene expression in this zygotic tissue.

Identifying genetic determinants of apomixis in dandelion *T. officinale* (Poster n°10)

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Engineering apomixis, the asexual reproduction via seeds, in crops would present several advantages for agriculture, such as pollen-independent seed production. This is particularly relevant since global warming will impact pollen viability and subsequently affect fruit and seed production. To obtain apomictic crops it is necessary to engineer three developmental components: (1) avoidance of meiosis, (2) fertilization-independent embryo development, and (3) autonomous endosperm formation. To date, avoidance of meiosis and fertilization-independent embryo formation have been successfully engineered in crops. However, the same is not true for autonomous endosperm (AE) formation. To introduce this trait, we first need to understand the molecular mechanisms underlying AE formation. In my PhD project, I aim to identify genetic determinants that regulate AE formation in dandelion which has been established as a model organism for the study of apomixis. Particularly, I will perform a comparative analysis between apomictic and sexually reproducing lines of dandelions. Then, we will use those molecular determinants to engineer asexual traits in crops. Thus, this work will contribute to the engineering of the last required developmental component of apomixis, aiming to achieve pollen independent seed formation in crops.

Post-pollination Reproductive Barriers Driving Speciation: Insights from *Torenia crustacea* (Poster n°11)

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Post-pollination reproductive processes in angiosperms involve various barriers that prevent interspecific hybridization and may be a key factor driving angiosperm speciation. However, the mechanisms underlying the evolution of post-pollination barriers between closely related populations and their contribution to speciation remain open questions. In this study, I addressed these questions using Torenia crustacea, which is widely distributed in Japan and is suitable for analysis of reproductive processes. Through genome-wide SNP-based population genomic analyses and inter-population crossing experiments, I identified endosperm (double fertilization product)-based reproductive barriers between populations from the Honshu Island and the Ryukyu Islands and demonstrated that these populations are reproductively completely isolated biological species. Furthermore, speciation was estimated to have occurred within an exceptionally short period of approximately 40,000 years. This rapid evolution of postzygotic reproductive barriers in *T. crustacea* may be driven by divergence in mating systems between selfing and outcrossing, and changes in past population size.

How to elucidate dosage compensation and parental conflict in *Silene latifolia* (Poster n°12)

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The parental conflict theory proposes that fathers maximize investment in current

offspring, while mothers distribute resources to balance investment between current and future progeny. In plants, this conflict is evident in seed development: while embryo maintains an equal maternal and paternal genome ratio, the nutritive endosperm tissue has 2:1 maternal-to-paternal ratio, favouring the maternal contribution. Despite progress in *Arabidopsis* and other plant models, the effect of polyploidy on parental resource allocation and embryo development remains poorly understood, particularly regarding perisperm growth. In *Silene latifolia*, a dioecious plant with heteromorphic sex chromosomes (2n = 24; XX females, XY males), parental conflict is expected to be mitigated since resource are stored in the perisperm rather than the endosperm. This suggests evolutionary older but effective strategy for maintaining maternal control over seed supply. To investigate whether polyploidy disrupts maternal control, we performed reciprocal crosses between diploid and tetraploid individuals. We observed that in maternal-excess crosses (4n mother × 2n father), most seeds underwent early abortion, whereas in paternal-excess crosses (2n mother × 4n father), some seeds abort late while others developed and appeared viable. We further examined seed size and embryo viability in these interploidy crosses using variable staining techniques. Our findings suggest that *Silene latifolia* provides a compelling system for studying the emergence of parental conflict in seed development. In particular, our results provide a new long-term direction to understand the mechanisms of parental conflict in the evolutionary young sex chromosome system such as *Silene latifolia*, and dioecious plants in general. Acknowledgement: This work was supported from the project TowArds Next GENeration Crops, reg. no.CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes nAmos Comenius.

Unravelling the influence of temperature on the switch from sexual to apomictic seeds through phenotypic plasticity (Poster n°13)

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Reproduction via seeds can occur sexually or asexually. In either case, plants produce seeds consisting of an embryo, the nourishing endosperm and the protective seed coat. The asexual pathway is called apomixis, a process of reproduction (almost) independent of sperm cells. This pathway can be separated into the following steps: (1) apomeiosis, the suppression of meiosis leading to diploid ovules, (2) parthenogenesis, the formation of an embryo without fertilization and (3) the formation of an endosperm either via pseudogamy (pollen are required) or autonomous endosperm (AE) formation. When drawing attention to the habitat of asexually reproducing species (animals as well as plants), it was observed that apomicts tend to colonize colder and more northern environments than their sexual relatives. Thereby, we aim to test a possible role of (cold) temperature as a switch from sexual to apomictic AE formation by making use of phenotypic plasticity of AE formation under different temperatures. I thereby aim to identify (epi-) genetic temperature-dependent determinants of AE formation in a variety of geographically distinct Arabidopsis thaliana ecotypes. Moreover, I will test the influence of temperature on the mode of reproduction on a natural apomict, *Boechera sp.*, which is a close relative of Arabidopsis thaliana. With this, we aim to study how sexual and asexual endosperms respond to changes in temperatures, and whether these responses can be adaptive.

The secrets of apomictic seeds: more sperms, less troubles (Poster n°14)

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Successful seed development in flowering plants relies on double fertilization, which gives rise to the embryo and its nutritive tissue, the endosperm. In most angiosperms, the endosperm genome maintains a 2:1 maternal-to-paternal ratio (2m:1p), critical for the proper regulation of imprinted genes and seed development. Deviations from this ratio, as seen in interploidy crosses, often result in seed failure. Apomictic plants exhibit remarkable flexibility by tolerating maternal genome excess in the endosperm (e.g., 4m:1p or 4m:0p). However, such deviations from the parental genome ratio may come at a cost. In our study of facultatively apomictic tetraploid Rubus species, we found that seeds with a balanced 2m:1p endosperm ratio were consistently larger, regardless of whether they originated sexually or apomictically. While apomictic seeds from tetraploid parents typically exhibit a 4x embryo and 10x endosperm, some frequently develop a 12x endosperm. This pattern suggests fertilization of the central cell by either an unreduced sperm cell or two reduced sperm cells, thereby restoring the 2m:1p balance. To investigate the genotype of the sperm cells contributing to endosperm development in apomictic seeds, we employed flow cytometric seed screening and microsatellite genotyping-by-sequencing simultaneously to every single seed. Our results confirmed that 12x endosperms in apomictic seeds arise exclusively from fertilization by two reduced sperm cells. Notably, we also detected cases of triparental endosperms, where two genetically distinct fathers contributed to the 12x endosperm formation.

Brassinosteroid-mediated environmental plasticity of seed development (Poster n°15)

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Plants are sessile organisms that have developed strategies to cope with various environmental challenges throughout their life cycle. In most natural accessions of *Arabidopsis thaliana*, temperature changes have been observed to influence seed traits, such as endosperm proliferation and seed size. However, it remains unclear of how seeds perceive temperature cues and regulate the temperature responses. Brassinosteroids (BRs), a class of plant hormones, are promising candidates in this process since we observed that seeds of BR mutants show attenuation in temperature responses when compared to wild-type seeds. Therefore, my PhD thesis aims to investigating the role of BR in regulating the developmental plasticity of seeds in response to temperature. In particular, I will focus on the study of the key BR effector transcription factor, BRASSINAZOLE-RESISTANT 1 (BZR1), which our observations suggest may be an important integrator of the temperature responses. By conducting transcriptomic analyses and CUT&RUN profiling of BZR1 targets, we aim to identify genetic determinants that are regulated by BZR1 during temperature changes. This will be coupled with biochemical and microscopy analyses to further examine its role during temperature responses. Moreover, since we have shown that BRs promote seed expansion in a manner dependent on the composition of the cell wall, I aim to understand whether seed coat cell wall compositions are modulated by different temperatures. This study will enhance our understanding of developmental

plasticity of seeds in response to temperature, thereby facilitating crop engineering to adapt diverse environmental conditions.

The carpel: A problem child we fail to grasp (Poster n°16)

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Gynoecia are rather complex, both morphologically and functionally. This inherent complexity has led to sorting them out, as Linnaeus did, into apparently meaningful categories (apocarpous and syncarpous) based on identifiable morphological relationships between their elementary units: the carpel. The latter is assumed to be the unit affected by evolutionary processes and developmental constraints through evolutionary times. This MSc research project consists of using a two-pronged approach (A and B) to investigate carpel structural components and their relationships across Rosidae - the subclass including the model species *Arabidopsis thaliana*. Among the three classical and ad hoc morphological sub-units (ovary, style, stigma) acknowledged, the style concentrates much on questioning, notably its 'closure'. A) Based on a series of mutants of *A. thaliana*, we investigate GRNs and cellular processes that can lead to style closure. This will allow us to pinpoint the relevance of different methods (anatomy, organogeny, functional study) to identify and interpret morphological structures properly. B) Following literature-based data relative to a series of gynoecial characters, we will provide a conceptual framework towards an understanding of the carpel structures, based on phylogenetic comparative methods.

Evolutionary insights into plant reproduction: the role of JAGGED-like zinc finger transcription factors (Poster n°17)

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Morphogenesis of flowering plant's reproductive organs involve tight regulation by gene regulatory networks which contribute to patterning processes requiring concerted cell division and expansion. In *Arabidopsis*, JAGGED (JAG) and its close paralog NUBBIN (NUB) are important transcriptional repressors regulating diverse aspects of polar growth of reproductive organs, including cell proliferation, expansion and anisotropic growth. Most likely, they act via protein interaction with the strong transcriptional repressor TOPLESS (TPL) via an Ethylene Response Factor (ERF), associated an amphiphilic repression-domain (EAR) motif. However, the function of these regulators in plants outside the angiosperms are unclear, and we aim to elucidate their evolutionary history and how they have contributed to reproductive development in other land plant lineages. Here, we provide a molecular evolutionary framework for land plant JAG-like genes showing their presence in all lineages, but a lack of EAR motifs in many genes outside the vascular plants. This suggests that JAG-like genes of Bryophytes and some Monilophytes differ in their molecular mechanism of action from the canonical JAG-like genes found in angiosperms. We further show that JAG-like genes are present in all land plants and that their expression in all land plant lineages is mainly found in sporophytic reproductive tissue. And lastly, we will introduce the recent results of our functional analysis of JAG-like genes from non-angiosperms.

A female in vivo haploid-induction system via mutagenesis of egg cell-specific peptidases (Poster n°18)

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Crop breeding schemes can be significantly accelerated by using (doubled) haploid plants. In vivo haploid induction has been applied in plant breeding for decades but is still not available for all crops and genotypes, and haploidization rates are generally very low. Therefore, methodological improvements to and new concepts for haploidization are required. Here, we report a novel system for the induction of haploid plants by mutating genes encoding egg cell-specific aspartic endopeptidases (ECSs). We show that after successful sperm–egg cell fusion, ECSs play a critical role to ensure male and female nucleus fusion after fertilization. The ecs1 ecs2 double mutant can induce haploids by both selfing and hybridization in *Arabidopsis* and ECS mutation is also capable of producing haploids in rice. In summary, our study develops a novel approach for maternal haploidization and provides new insights into the molecular basis of fertilization.

The role of trehalose 6-phosphate and *TREHALOSE-6-PHOSPHATE SYNTASE* in the evolution of land plants (Poster n°19)

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The low abundance signaling sugar trehalose 6-phosphate (Tre6P) has gained more and more attention as an important regulator of sucrose levels, influencing metabolic fluxes and plant development. Both Tre6P and its synthesizing enzyme TREHALOSE-6-PHOSPHATE SYNTHASE (TPS) have been shown to regulate metabolism, flowering and embryogenesis, primarily in angiosperms. TPS proteins, however, are present throughout the whole green lineage, not just flowering plants, and the number of them has increased with the transition to land. Their function in non-vascular plants and algae has barely been researched before. This project aims to modulate sugar and Tre6P levels to observe changes in metabolite and phenotype in extant representatives of land plants: bryophytes, and the algal sister lineage of land plants: Zygnematophyceae. We want to understand, how Tre6P and TPS might have helped guiding transition from water to land and how their function has developed from unicellular algae to be regulators of complex multi-cellular processes like flowering and generational change.

Evolution of the *miR156/529-SPL* network in land plants (Poster n°20)

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During the evolution of streptophytes the reproductive dependencies between sporophytes and gametophytes have changed. Extant lineages of land plants show different nuances of interdependencies between the two life stages. Here, bryophytes and angiosperms constitute examples for the most extreme variations on the spectrum: a fully dependent sporophyte (bryophytes) and a fully dependent gametophyte (angiosperms). In contrast, different fern lineages hold more variation with some ferns having independent sporophyte and gametophyte stages and others showing

gametophytic dependency on the sporophyte. The molecular chassis underpinning the degree of dependency is scarcely understood. The initiation of reproduction and with that the shift from gametophytic to sporophytic tissue or vice versa is, among others, controlled by the microRNA family miR156/529 in bryophytes and angiosperms. Their evolutionary history and dynamics of targeting SPL genes by miR156/529 is less investigated. We found that miR156/529 share a common ancestry, and miR529 was lost multiple times during the evolution of embryophytes. The miR529-SPL module was likely functionally replaced in those lineages by the miR156-module. Constantly, lineages that have miR156 and miR529 members, targeting of SPL is predicted to be redundant. Overall, our targeting analyses suggest a rapid target turnover, which likely accommodates the strong lineage-specific radiations of the SPL family. Having established a first inference on the evolutionary trajectory, we are currently diving deeper into the variation of targeting and expression of target genes in a diverse set of ferns with different gametophyte-sporophyte dependencies to understand the behavior of miR156/529-SPL module in different gametophyte-sporophyte dependencies.

Evolution of reproductive coordination in *Azolla* (Poster n°21)

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The relationship between the water fern *Azolla* and its nitrogen-fixing cyanobacterial symbiont (cyanobiont) is unique among land plants. The latter is vertically inherited to facilitate a perpetual, co-dependent symbiosis. The cyanobiont is maintained in an extracellular cavity in the fern's leaves. *Azolla* predominantly reproduces asexually, via abscission of leaves. It can switch to sexual reproduction through spores, in which case the cyanobiont is transmitted maternally through the megasporocarp. We seek to identify the molecular network responsible for transmission of the cyanobiont in both pathways. Sexual reproduction will be induced with far-red light. Tissue from various developmental stages of megasporocarps, tissue involved in cyanobiont transmission during vegetative growth, and older tissue that is no longer transmitting will be sampled to perform dual-RNA sequencing of all six *Azolla* species and their cyanobionts. Differentially expressed genes between reproductive modes and developmental stages will be identified and network analyses performed to identify the reproductive network. Additionally, we will conduct population genomics of *Azolla filiculoides* to identify selective pressures affecting the reproductive signalling network and transmission of the cyanobiont. The aim of this project is to identify how the reproductive network of the heterosporous ferns from the genus Azolla was changed and co-opted to co-develop with cyanobacterial transmission and allow for the only permanent symbiosis observed in land plants other than the chloroplast.

***Open Flower*: A blooming model for teaching, outreach and research (& Poster n°22)**

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See the third talk of *Session 8: Bioinformatics solutions to harness the EvoDevo Data* for abstract

Comparing expression of orthologous genes between different plants using *GenXBrowser* (Poster n°23)

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Comparing gene expression across plant species is crucial for understanding functional conservation and divergence, but it remains challenging due to technical complexities. We present *GenXBrowser*, a user-friendly web-based tool for interactive exploration of cross-species transcriptomic data. Using *Capnoides sempervirens* and *Papaver somniferum* from the RanOmics project, we demonstrate how *GenXBrowser* facilitates comparison of 12 tissue types by filtering orthogroups. Analysis of Orthogroup 70, containing chlorophyll a-b binding proteins, revealed conserved high expression in photosynthetic tissues (young/mature leaves) across both species. Notably, *P. somniferum* exhibited additional expression in petal anthesis tissues, suggesting potential functional divergence. *GenXBrowser*'s intuitive interface allows researchers to easily identify such expression differences, which may underlie ecological adaptations or biosynthetic variations. By reducing technical barriers, *GenXBrowser* empowers non-computational biologists to explore complex transcriptomic datasets and generate hypotheses about gene function evolution across plant species.

The secreted redox sensor roGFP2-Orp1 reveals oxidative dynamics in the plant apoplast (Poster n°24)

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Specific generation of reactive oxygen species (ROS) is important for signalling and defence in many organisms. In plants, different types of ROS perform useful biological functions in the extracellular space (apoplast) supporting processes such as polymerisation and cell wall remodelling. In addition, increased formation of extracellular superoxide and hydrogen peroxide occurs during immune responses. Our current knowledge of apoplastic ROS dynamics is based on luminescence assays, ROS staining methods and indirect evidence via changes to intracellular redox balance. However, dynamic monitoring of extracellular redox processes in vivo remains difficult. Using two evolutionary distant land plant model species, the moss *Physcomitrium patens* and the flowering plant *Arabidopsis thaliana*, we test whether the genetically encoded redox biosensor roGFP-Orp1 can be used to assess extracellular redox dynamics. We found that secreted roGFP-Orp1 can inform about local diffusion barriers, as well as protein cysteinyl oxidation rate in the apoplast after pre-reduction. Observed re-oxidation rates were surprisingly slow within the range of hours. Comparing *A. thaliana* to *P. patens*, we found faster sensor re-oxidation that increased after triggering an immune response. Our data indicate differences in extracellular oxidative processes between species and within a species, depending on immune signalling and RBOH dependent ROS production.