Abstracts

Hormonal control and evolution of branching forms in mosses

Coudert, Yoan

Branching patterns are a primary determinant of plant architecture and strongly impact on productivity by regulating light harvesting potential and resource allocation. Plants colonized land over 450 million years ago, and underwent architectural diversification in the haploid (gametophyte) and diploid (sporophyte) genetic stages of the life cycle independently. Although similar branching mechanisms evolved in both genetic stages, our functional understanding of branching is limited to diploid flowering plant models such as *Arabidopsis.* To test whether the same molecular cues regulate similar lateral branching mechanisms that have evolved independently, we undertook a computational and genetic analysis of branching patterns in the haploid leafy shoot of a moss- *Physcomitrella patens*. We show that a simple model co-ordinating the activity of shoot tips across the plant can account for the branch distribution, and that three known hormonal regulators of branching in flowering plants generate the pattern. Importantly, these cues may be integrated via a novel mechanism in moss.

Whilst ancient hormonal cues have similar functions in distantly related lineages, the mechanisms underpinning the diversification of branching forms observed in nature remain largely unknown. In mosses, branching forms are hypothesized to have diversified in response to a single driver- the displacement of reproductive structures away from the main shoot apex to lateral positions, known as pleurocarpy. To test the above hypothesis we have considered *Physcomitrella patens* as an evolutionary starting point and characterized the architectures of 175 moss species. Using ancestral character state reconstruction, we have reconstructed the evolutionary history of moss architecture and identified two stages of diversification. Our results refute the hypothesis that pleurocarpy was the sole driver to moss diversification and pinpoint the nature of developmental change driving the radiation of branching forms in the second largest group of land plants. Building on our functional work, these results provide a framework to explore the hormonal control of branching form evolution in the moss lineage.

CLAVATA-Like Receptors are Ancient Regulators of SAM Identity and Cell Division Plane

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Plant shoot morphology is determined by patterning events that occur at the shoot apical meristem (SAM), but how developmental networks function in the SAM to produce the wide range of extant plant morphologies is unknown. The gametophytic shoot (gametophore) of the moss Physcomitrella patens is initiated by a series of oblique, asymmetric cell divisions that produce a single tetrahedral apical cell. This cell continuously grows and divides to pattern leaf (phyllid) positioning while replenishing itself throughout shoot development, making it equivalent in function to an angiosperm SAM. The moss SAM thus provides simple, genetically tractable system for study where the processes of cell growth, cell division, and stem cell homeostasis are tightly coordinated to ensure proper morphogenesis. Previous research discovered a high degree of overlap between the transcriptomes of moss and angiosperm SAMs, suggesting that the equivalent functions fulfilled by these structures are likely regulated by homologous gene regulatory networks. We test this hypothesis by investigating the function of a set of genes upregulated in the moss SAM that are orthologous to Arabidopsis thaliana receptor kinases of two clades, the first containing CLAVATA1 (CLV1) and the second containing RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2). Mutants of these receptor kinase genes display varied but abnormal division planes leading up to SAM initiation and form numerous ectopic meristems along the gametophore axis. We demonstrate that these classical regulators of stem cell maintenance have a conserved role regulating stem cell identity in the Physcomitrella gametophore, as well as a novel role in orienting plant cell division planes.

PGGT-I and ROP modules contribute the development of multicellularity in plants

Bao, Liang

One of the most important innovations of life on earth is the transition to multicellularity from unicellular ancestors, but little is known about barriers or drivers of this transition. Here we show that control of multicellularity in the moss Physcomitrella patens is through PGGT-I and ROPs. PGGT-I (protein geranylgeranyltransferase-I) is composed of alpha and beta subunits, and all four ROPs in the moss Physcomitrella patens belongs to type I ROPs, terminating with a canonical CaaL box, which is the PGGT-I target sequence. Previously, studies by the Bezanilla lab showed that transient knockdown of ROPs resulted in defects in cell adhesion and cell polarity, and studies by our lab showed that knockouts of the PpGGB gene, which encodes for the geranylgeranyltransferase-I beta subunit, produced single-cell like plants. To systematically study the relationship of cell adhesion and the PGGT-I and ROP pathway, we have generated stable inducible knockdown lines of ROPs by artificial micro-RNA (amiRNA), which further confirmed the essential function of ROPs in cell adhesion and cell polarity. Moreover, while overexpression of ROP in the ggb mutant background rescued cell adhesion defects in ggb mutants, making the single-celled ggb plants form filamentous cells, overexpression of a mutant form of ROP, changing from Caal to the non-prenylation target SaaL box, did not rescue. Similarly, while overexpression of ROP in the wild type background causes filamental protonemata to become round cells, overexpression of a mutant form ROP terminating with SaaL box did not affect any cell morphology. Using SEM and live imaging, we have found that the loss of cell adhesion is caused by uncontrolled and uncoordinated cell expansion, which could disrupt the cell walls. Thus, we propose that the PGGT-I and ROP pathway contributed to the evolution of multicellularity in plants by controlling coordinated cell expansion among neighboring cells.

Genetic pathways controlling meristematic activity in hornwort-sporophytes

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One major change that accompanied the evolution of embryophyte land plants from a haplontic ancestor was the elaboration of the sporophyte and parallel reduction of the gametophyte phase. Flowering plants have established complex branched sporophytes, which grow through the continuous activity of a multicellular meristem located at the tip of the shoot. Genetic developmental pathways controlling the activity of this shoot apical meristem have been researched extensively in flowering plants. In contrast, bryophytes, the most basal group of extant land plants, have subordinate, unbranched, monosporangiate and upright sporophytes that remain attached to the gametophyte generation. Bryophyte-sporophytes exhibit multicellular meristems that contribute to sporophyte growth, most notably the intercalary meristem of moss-sporophytes and the basal meristem of hornwort-sporophytes. Yet, regulatory mechanisms controlling the activity of these meristematic regions are not known. Comparison of regulatory gene networks controlling sporophyte development could help to resolve the evolutionary-developmental trajectory between bryophyte-sporophytes and the more complex sporophytes of vascular plants. Therefore, we are currently working to provide a detailed account on the regulatory mechanism governing sporophyte development in bryophytes. To this end, we established transcriptomic profiles for five different sporophyte tissues of the hornwort *Anthoceros agrestis*, using laser-assisted microdissection coupled with RNA-sequencing. Analysis of differential gene expression across the five tissues allowed us to establish a first hypothetical model for the regulatory mechanisms governing sporophyte development in hornworts. Future work will focus on describing fine-scale spatial expression of proposed candidate genes and testing their functional role using reverse genetic approaches.

Seasonal regulation of sexual reproduction in Physcomitrella patens

Hicks, Karen

Sexual maturity occurs at a specific time of year in a wide range of organisms, including both plants and animals, significantly impacting both reproductive capacity and agricultural production. Light and temperature serve as the primary seasonal cues for these organisms, and the mechanisms by which these cues regulate the transition from vegetative to reproductive development have been and are being addressed in many angiosperm species. We are using the moss Physcomitrella patens to probe the evolutionary origin of seasonal regulation in land plants, with the long-term goal of determining if seasonal regulation evolved in the common ancestor of all land plants, or if it arose separately in distinct land plant lineages. A third model would involve conserved upstream pathway components coupled to a novel downstream regulatory module that induces reproductive development in response to proper seasonal cues. In order to distinguish between these alternatives, we are utilizing natural variants that differ in their responses to seasonal cues in order to identify candidate genes involved in seasonal regulation in Physcomitrella. While the Gransden reference strain is highly responsive to photoperiodic and temperature cues, we have identified several accessions that lack strong seasonal control of sexual reproduction and thus differ in their timing of gamete development in response to temperature and/or daylength cues. We are currently using a combination of whole-genome re-sequencing and RNAseq transcript profiling to identify genes that may be involved in reproductive timing in response to environmental cues to distinguish between our hypotheses. Enrichment analysis of our current dataset supports conservation of upstream pathway components between P. patens and angiosperms.

PpCDKA controls light signaling in Physcomitrella patens

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In cell cycle progression, cyclin-dependent kinase1 (CDK1) is one of the key factors and is essential for survival in mammals and yeasts. In flowering plants, the mutant of *Arabidopsis CDKA;1*, an ortholog of *CDK1*, shows severe growth defects.

The moss *Physcomitrella patens* has two copies of *CDK1* (*PpCDKA*) in the genome. To investigate function of PpCDKA, we constructed *PpCDKA*-null mutants (*ppcdka*) and unexpectedly found that protonemata and gametophores in *ppcdka* are viable and look healthy. This suggests that some characteristics of PpCDKAs in *P. patens* are markedly different from those of other organisms.

Therefore, we study its phenotype and found that several photo-responses such as phototropism, and chloroplast photo-relocation movement were impaired in the mutant. We especially focus on chloroplast photo-relocation which is important for efficient photosynthesis and photo-damage avoidance. Although several components related to chloroplast photo-relocation movement have been found in plants, the overall pathway is still not clear. A new model of chloroplast photo-relocation pathway including PpCDKA will be presented.

The role of volatile organic compounds in antagonistic selection in the moss Ceratodon purpureus

Kollar, Leslie

A central goal in evolution is to understand the mechanisms that maintain genetic variation for fitness. Across much of the tree of life, males and females are clearly differentiated in many non-reproductive traits, presumably because selection favors different trait optima in each sex. Thus, an allele that increases fitness in one sex can be deleterious in the opposite sex, causing genetic conflict. The role of genetic conflict in maintaining variation for fitness depends upon the degree to which males and females respond similarly to an allelic substitution (i.e., the cross-sex correlation) and the difference in optimum phenotypes between the sexes, both poorly understood quantities. Here, I estimated the cross-sex correlation for several life history traits in the moss, Ceratodon purpureus using a common greenhouse experiment with 46 haploid-sibling families, each comprising three male and three female offspring. I focused on sexual dimorphic volatile organic compound (VOC) production. Analogous to flowering plant-pollinator mutualisms, female C. purpureus gametophytes emit abundant VOCs to attract sperm-dispersing microarthropods, which significantly increase fertilization rates in moss. Male mosses produce fewer VOCs than female mosses, suggesting that VOC production may be costly. The cross-sex correlations across all traits were less than one but greater than zero, suggesting that intersexual genetic conflict can maintain genetic variation for fitness. Next I plan to conduct competitive mating experiments in controlled mesocosms to identify traits linked with female and male reproductive success.

Molecular basis of convergent evolution: parallel reduction of the sporophyte phase in mosses

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The phenomenon of convergent evolution, the repeated evolution of traits in independent lineages, provides ideal replicates to test for constraints on the trajectory of evolutionary processes. We aim at investigating the molecular mechanisms of convergent evolution by studying the evolutionary-developmental mechanisms underlying the repeated evolution of reduced sporophyte phenotypes in the moss family Funariaceae.
In bryophytes the dominant haploid gametophytic phase alternates with a diploid sporophyte phase. While the gametophyte remains largely unchanged within Funariid mosses, the morphology of the sporophyte differs drastically. Recent research on the phylogeny of the family has shown that highly reduced sporophytes evolved multiple times independently. This repeated morphological reduction together with the simple structure of the sporophyte phase and their amenability for reverse genetic work makes the Funariaceae family an ideal model system to study convergent evolution in a set of closely related species.
To address the question how reduced sporophytes are established we gathered transcriptomic data of four developmental stages of sporophytes from Funaria hygrometrica and Physcomitrella patens. While F. hygrometrica represents the complex sporophyte phenotype, the sporophyte of P. patens is reduced to a simple spherical capsule without dehiscence mechanisms. We identified orthologous genes between the two species and compared their expression patterns during sporophyte development. We were able to identify a set of candidate genes potentially responsible for the radically different sporophyte phenotypes. Furthermore, we used regulatory network analysis to recover conserved regulatory domains of sporophyte development and to assess in what extent rewiring or heterochronic changes have contributed to the evolution of divergent sporophyte phenotypes. In future work we will use laser capture microscopy assisted RNA sequencing and in-situ hybridization to more specifically assess differential expression of genes over sporophyte development in the two species. Additionally, we will use reverse genetics to verify the putative function of candidate genes. Combining the toolbox of fine-scale

Studies on the dynamics of polarized growth in mosses

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Bryophytes were the first plants to emerge from water life and colonized the earth over the time; the moss Physcomitrella patens has become an excellent model to plant biology and genomics. Mosses developed polarized growth to anchor to substrate, and this strategy has been conserved among angiosperms during pollen tube development and radical hair growth.
In this study we used fluorescence microscopy under in vitro culture conditions to evaluate the dynamic of the secretory vesicles during protonemata tip polar growth and the apical cell division. Regarding to the apical cell division, we detected a redistribution of the secretory vesicles along the apical cells. Furthermore, a decrease on the oscillatory growth during cell division was observed. In order to get insight into the role of intra and extra cellular reactive oxygen species (ROS), specific detection was monitored during protonemal growth. Specific patterns corresponding to internal ROS signals were detected, and also extracellular ROS just in the apical zone. We propose that ROS could be participating in the oscillation of hydrogen potential that has been reported in angiosperms radical polar growth (Cárdenas, 2009).
We thank to Instituto Politécnico Nacional, Secretaría de Investigación y Posgrado, SIP 2018, COFAA, CONACYT and Moss2018 for financial support.

Efficient Modular CRISPR-Cas9 System in P. Patens

Darren Mallett, Mingqin Chang, Xiaohang Cheng, Magdalena Bezanilla

CRISPR-Cas9 has been shown to be a valuable tool in recent years, allowing researchers to precisely edit the genome using an RNA-guided nuclease to initiate double-strand breaks. In the moss Physcomitrella patens, the classical RAD51-mediated homologous recombination pathway has been used for genome editing. However, there are limitations to using homologous recombination. In particular, the stable integration of unwanted exogenous sequences into the genome, such as an antibiotic resistance cassette, upstream or downstream of a gene could potentially alter gene expression. Additionally, it is challenging to edit multiple genes simultaneously. CRISPR-Cas9 provides an opportunity to efficiently edit the genome at multiple loci using a simple transient transformation in P. patens, thereby eliminating the need to stably incorporate a selection cassette. Here we describe a new CRISPR-Cas9 vector system based on vectors previously developed for rice (Miao et al., 2013). This vector system does not rely on gene synthesis. Instead, protospacers are synthesized as complementary oligos that are ligated into an entry vector taking advantage of type IIS restriction enzyme cut sites. Using Gateway technology, multiple guide RNA expression cassettes are seamlessly transferred to a destination vector that also expresses the Cas9 enzyme. Our system enables simultaneous targeting of up to 12 genomic sites in one transformation. Co-transformation with a DNA donor homology plasmid also allows particular changes to be integrated, such as fusions with DNA encoding for fluorescent proteins. Furthermore, a homology plasmid harboring a “stop codon cassette” ensures a null allele with near-effortless genotyping using the homology-directed repair pathway.

The Physcomitrella patens gene expression atlas

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In the context of the US Department of Energy's Joint Genome Institute (DOE JGI) Plant Flagship Gene Atlas project, a large scale RNA-seq expression data set was generated for Physcomitrella patens.

Our in-house RNA-seq data management pipeline (Perroud et al., 2018)although this method has been used to help the analysis of specific perturbations, no overall reference dataset has been established yet. In the framework of its Gene Atlas project, the Joint Genome Institute selected P. patens as a flagship genome, opening the way to generate the first comprehensive transcriptome dataset for this moss. The first round of sequencing described here is composed of 99 independent libraries spanning 34 different developmental stages and conditions. Upon dataset quality control and processing through read mapping, 28,509 of the 34,361 v3.3 gene models (83% was used to analyse the 99 RNA-seq samples generated from 34 different developmental stages and growth conditions, supplied by 13 different laboratories. Upon dataset quality control and processing through read mapping, 28,509 of the 34,361 v3.3 gene models (Lang et al., 2018)Physcomitrella patens, comprised approximately 2000 unordered scaffolds. In order to enable analyses of genome structure and evolution we generated a chromosome-scale genome assembly using genetic linkage as well as (end were detected to be expressed across the samples. Subsequently, differently expressed genes (DEGs) were called for 50 experiment comparisons. The analysis of the three most distinct and abundant P. patens growth stages, protonema, gametophore and sporophyte, made it possible to define both general transcriptional patterns and developmental stage-specific transcripts. Metabolic experiments and hormone treatments as well as inter laboratory comparison provided transcriptome-wide Gene Ontology (GO) term trends. The second set of 72 samples, covering another 25 stages and perturbations, has been sequenced and is currently being processed.

To ease public access to this abundant data, we developed the interactive web tool, PEATmoss (Physcomitrella Expression Atlas Tool). It facilitates gene expression visualization among multiple samples and genes in an interactive way. So far the JGI Gene Atlas RNA-seq data as well as selected previously published RNA-seq and microarray data are integrated in the tool. As more Physcomitrella patens expression data becomes available, it will included in PEATmoss.

Lang, D., Ullrich, K. K., Murat, F., Fuchs, J., Jenkins, J., Haas, F. B., … Rensing, S. A. (2018). The Physcomitrella patens chromosome-scale assembly reveals moss genome structure and evolution. *The Plant Journal. 93*(3), 515–533. https://doi.org/10.1111/tpj.13801

Perroud, P.-F., Haas, F. B., Hiss, M., Ullrich, K. K., Alboresi, A., Amirebrahimi, M., … Rensing, S. A. (2018). The Physcomitrella patens gene atlas project: large scale RNA-seq based expression data. *The Plant Journal*. https://doi.org/10.1111/tpj.13940

Assembly of the moss genome Funaria hygrometrica using Nanopore and NGS data

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The draft genome of the model moss Funaria hygrometrica was de novo assembled using 40 GB of Nanopore and 39 GB of Illumina NGS data. The genome assembly yielded a total length of 326,856,579 bp, with a contig N50 of 1.5 MB, which are distributed among 687 contigs with length longer than 2,000 bp. A preliminary clustering of the contigs to chromosomal linkage groups was made based on Hi-C result. The sequence depth of the genome was estimated to be >100 X on both data, since the estimation of the genome size is 362 Mb based on the 17-mer analysis of the NGS data. The initial assembly was built with SMARTdenovo using the Nanopore reads that corrected by Canu. The resulting contigs were then verified by the NGS reads using the GATK IndelRealigner and Pilon. The assembled genome of Funaria holds 871 homologues of 1440 total single-copy orthologs composing the BUSCO database. The repeated and low complexity regions of the genome were identified via RepeatModeler and were soft-masked via RepeatMasker. Short reads and assembled transcripts of Funaria transcriptome generated by NextSeq Mid-output 500 were aligned to the soft-masked genome via HiSAT2 and Gmap aligner, respectively. Braker was used for annotation through short reads and the outcome of that was compared to Gmap annotation results. The overlaps between Braker and Gmap were identified via Bedtools and the outcome was filtered for keeping only the complete overlaps between them. Ultimately, 30,181 multi-exonic genes with average size of 682 bp were identified. This final gene set was used for functional annotation via EnTAP and was further filtered for contamination and mono-exonic genes.

BRY"O"MICS: Application of high-sensitive and high-throughput molecular tools to disentangle the mechanisms of heavy metals accumulation and tolerance in mosses: epigenetic and transcriptomic approaches

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Current knowledge on the mechanisms used by plants to deal with environmental stress is mostly derived from tracheophytes. However, bryophytes due to their phylogenetic position between green algae and tracheophytes, are especially interesting organisms to unravel the complexities of the plant-environment interactions from an evolutionary perspective. Current evidence suggests that epigenetic changes allow angiosperms to respond to environmental stress. The role of epigenetics in the phenotypic variation of ecologically important traits in bryophytes is not as well established.

Exposure to heavy metals (HM) imposes a strong environmental pressure to plants and yet bryophytes are able to thrive and even specialize to live in highly contaminated environments. Thus, BRY"O"MICS aims to provide a deep understanding of the mechanisms underlying the existence of phenotypic variability for HM tolerance and hyperaccumulation in bryophytes. We collected two ecologically different moss species, Scopelophila cataractae (a "copper-moss" restricted to HM enriched habitats) and Ceratodon purpureus (a cosmopolitan species occurring in a variety of substrates) in the field. We also cultured them in the laboratory under different HM treatments. Reduced representation bisulfite sequencing (RRBS-seq) and RNA sequencing (RNA-seq) were used to build the methylome and transcriptome profiles of these species and gain insight in their ability to accumulate and deal with these pollutants.

Preliminary results show that both species responded significantly to HM exposure. Gametophytes of S. cataractae were smaller in field populations growing on the most contaminated sites; C. purupureus showed significant population and sex specific inhibition of growth and increased oxidative damage in response to Cd and Cu in the laboratory.

We expect to find correlated changes in DNA methylation and gene expression patterns in response to HMs in both species (e.g. population and/or sex-dependent changes), as well as interspecific differences in the molecular mechanisms used to cope with Cd and Cu.

High-density linkage map enables chromosome-scale assembly of the Marchantia polymorpha genome.

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M. polymorpha has recently become a prime model for investigation in the fields of cell and developmental biology, evo-devo research, synthetic biology, and evolutionary ecology. Due to its critical phylogenetic position and the assumption that the earliest land plants were liverwort-like, it is frequently used to investigate how plants colonized and adapted to the terrestrial environment and how increasing overall complexity has evolved throughout the land plants. In 2017, the genome of M. polymorpha was released but the 230 Mb large genome is composed of 2957 scaffolds, making the analysis of (epi)genome structure and the application of classical genetic mapping difficult.

Here we present a chromosome-scale assembly of the M. polymorpha genome using a high-density linkage map with a total map length of 712 cM and an average marker density of 0.1 cM. Using the linkage map, we arranged 90% of the scaffolds of the v1.3 assembly into eight linkage groups corresponding to the eight autosomes of M. polymorpha covering 208 Mbp of the genome. We found that overall genome structure of M. polymorpha is strikingly different from that of the model moss Physcomitrella patens. Specifically, recombination rates vary considerably across the chromosomes and correlate both with genetic polymorphisms and the presence of repeats/centromeres. Our analyses also revealed large misassemblies in the draft genome, which we corrected using long-read sequencing technologies. Altogether, our chromosomal-scale genome assembly opens up new avenues in M. polymorpha research by making detailed analysis of genome structure and classical genetic mapping approaches, forward genetic screens, feasible.

Experimental tools for the new hornwort model species, Anthoceros agrestis.

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Recently, a new bryophyte model species has received increased attention – the hornwort. Analyzing the hornwort genome will help to resolve fundamental questions of plant evolution and developmental biology, in general, and unique aspects of hornwort biology, in particular. However, having a fully sequenced genome does not fulfill all requirements of a well-established model organism. Here we report the advancements concerning culturing, genetic transformation and laser capture microscopy assisted small-scale gene expression profiling (LCM RNA-seq) of the model organism Anthoceros agrestis achieved over the last year. In particular, we will elaborate on the tractability of the system for experimental research by first introducing main features of its nuclear genome and present our results on the protoplastation, transient transformation, and regeneration of hornwort tissues. Finally, we will show some of our first results regarding the origin and evolution of the hornwort basal meristem using LCM assisted RNA-seq. Altogether, these achievements show that Anthoceros agrestis is becoming, step by step, a better-studied model organism for developmental, molecular, genomic, and genetic studies.

Do bryophytes make anthocyanins? A study in scarlet.

Bryan Piatkowski and Karn Imwattana

Duke Biology

Flavonoids are secondary metabolites produced throughout the Viridiplantae that have a broad range of functions and perhaps facilitated the transition of early plants onto land. One class of flavonoids, the anthocyanins, are best known from flowering plants but have been reported from several lineages of bryophytes. For example, many species in the moss genus Sphagnum produce red pigments called sphagnorubins that have been described as either anthocyanins or derived from anthocyanins. In flowering plants, the biosynthetic pathway for the production of anthocyanins is well-characterized. Using a phylogenetic approach, we attempted to identify putative bryophyte orthologs to known anthocyanin biosynthetic genes. Probabilistic homology searches were conducted using twelve Viridiplantae genomes and phylogenetic trees were inferred for each gene family in the anthocyanin biosynthetic pathway. Contrary to previous studies, we find that the bryophytes possess only upstream genes in the pathway. Liverworts possess more genes in the pathway than mosses do. These results provide insight into the evolutionary history of the anthocyanin biosynthetic pathway and suggest that, at the enzymatic level, the production red flavonoid pigments in bryophytes may be an example of convergent evolution.

Characterization of two conserved mycorrhizal signaling genes in Physcomitrella

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Arbuscular mycorrhizal and root-nodule symbiotic interactions in land plants are controlled by a conserved plant signal transduction pathway. Often, many genes in this pathway are absent in distinct species or whole clades of non-host plants, such as the Brassicaceae. In particular, the calcium/calmodulin-dependent protein kinase (CCaMK) and its direct target Interacting Protein of DMI3 (IPD3), which are both required for symbiotic infection, are highly conserved in host lineages and absent in non-hosts. Interestingly, there are CCaMK and IPD3 homologs present in Physcomitrella patens despite the non-mycorrhizal habit of most mosses reported, suggesting a divergence of CCaMK/IPD3 function in mosses. We used biochemical and genetic approaches to characterize two CCaMK homologs and one IPD3 homolog from P. patens. Biochemical analyses of recombinant proteins have indicated that one CCaMK homolog has retained Ca^{2+}/CaM-regulated kinase activity and a physical interaction with IPD3, whereas the other CCaMK homolog has not. Both CCaMK variants were expressed in Medicago truncatula roots to determine if they rescue symbiotic defects; similarly, the CCaMK displaying canonical activity rescues rhizobial and mycorrhizal infection, whereas the other does not. Expression of gain-of-function variants of CCaMK and IPD3 in P. patens both induce the ectopic development of brachycytes, a stress response associated with drought in mosses. We also show that these tissues contain elevated levels of abscisic acid and Late Embryogenesis Abundant transcripts. The significance of CCaMK/IPD3 activation and drought-responses in mosses will be discussed.

Understanding the diversity and genetic basis of hornwort-cyanobacteria symbiosis

Fay-Wei Li, Jessica Nelson, Juan Carlos Villarreal

Plant symbiosis with nitrogen-fixing cyanobacteria is a unique form of mutualistic association that has independently evolved in diverse lineages including a few species of bryophytes, ferns, cycads, and one small genus of flowering plants. Compared to other nitrogen-fixing microbes, cyanobacteria are generally less dependent on the plant host, and therefore could be an ideal partner for engineering symbiotic nitrogen fixation into crop plants.

However, our current understanding of plant-cyanobacteria symbioses is rudimentary. The phylogenetic diversity of cyanobionts has been largely unexplored, and most genetic research has solely focused on the model cyanobiont Nostoc punctiforme; the plant genes involved in cyanobacterial symbiosis remain unknown.

Here I will present our ongoing work to characterize the hornwort cyanobiont diversity using PacBio amplicon sequencing. I will also discuss the putative symbiosis genes we identified in the model hornwort Anthoceros agrestis by RNA-seq analyses.

Quantifying moss associated nitrogen fixation in Alaska

Stuart, Julia

The future carbon (C) sequestration potential of the Arctic and boreal biomes, currently the largest terrestrial C sink globally, is linked to nitrogen (N) cycling, and changes in plant communities and N cycling can constrain or accelerate positive feedback loops between carbon (C) storage and climate. Pristine environments in Alaska have low anthropogenic N deposition (<1 kg N ha^{-1} yr^{-1}), and the main source of new N to these ecosystems is through previously understudied N_2 fixation from microbial communities on mosses. Despite the importance of moss associated N_2 fixation, the relationship between moss species, microbial communities, and fixation rates remains ambiguous. In the summer of 2016, the fixation rates of 34 moss species from sites around both Fairbanks and Toolik Lake were quantified using $^{15}N_2$ incubations. Subsequently, the microbial community and moss genome of the samples were also analyzed by collaborators. The most striking result is that almost all sampled moss genera fixed N, including well-studied feather mosses such as Hylocomium splendens and Pleurozium schreberi as well as less common but ecologically relevant mosses such as Aulacomnium spp., Dicranum spp., Ptilium crista-castrensis, and Tomentypnum nitens. Across all samples, fixation rates ranged from 0-55.6 μg N g^{-1} moss d^{-1}. Additionally, there were significant differences in fixation rates between broad categories of host mosses, such as acrocarpous and pleurocarpous mosses. Given this, linking variation in N_2 fixation rates to microbial and moss community structures can be helpful in predicting future trends of C and N cycling in northern latitudes. Vegetation changes, alterations in downstream biogeochemical N processes, and anthropogenic N deposition could all interact with or alter moss associated N_2 fixation, thereby changing ecosystem N inputs. Further elucidation of the species level signal in N_2 fixation rates and microbial community will augment our knowledge of N cycling in northern latitudes, both current and future.

Determining the genetic and environmental factors underlying mutualism within a peatmoss – N2 fixing bacterial association

David J. Weston[1], Alyssa Carrell[1], Dale Pelletier[1], Jeremy Schmutz[2], Dušan Veličković[3], Rosalie K. Chu[3], Christopher R. Anderton[3], Katherine B Louie[2,4], Trent R. Northen[2,4]

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The importance of plant-microbiome systems on terrestrial carbon and nitrogen processes is perhaps most pronounced in Sphagnum dominated ecosystems, which occupy 3% of the Earth's land surface yet store approximately 25% of terrestrial carbon as recalcitrant organic matter (i.e., peat). Together with associated N_2-fixing microorganisms, Sphagnum contributes to substantial peatland nitrogen inputs. Sphagnum growth and production (carbon gain) depends, in part, on a symbiotic association with N_2-fixing, diazotrophic microbes. Under changing environmental conditions, a central question about these ecosystems is whether the Sphagnum-diazotroph symbiosis will maintain its beneficial interaction, or will it shift to neutral or even antagonistic interactions that ultimately influence peatland carbon gain and storage. To begin to address this question, we are initiating a 5-year project using field-scale warming manipulations, synthetic communities, genotype-to-phenotype associations, and metabolic characterization to address two overarching hypotheses, 1) Sphagnum host and diazotroph genetic variations play a key role in determining the environmental tipping point of beneficial symbiosis (i.e., environmental disruption), and 2) the surrounding microbiome can further adjust the tipping point through facilitation, competition, and antagonism. Results from our field manipulation study show that warming decreased Sphagnum associated microbial diversity ($p<0.05$), and that N2 fixing diazotrophs shifted from diverse communities to those dominated by Nostocaceae (from 25% in unheated samples to 99% in warmed samples). To provide a fundamental understanding of the field results, we are developing resources for synthetic communities. We now have draft genomes of 15 Sphagnum species and (re)sequencing of a 200-member pedigree was recently completed and being used for genetic map construction. On the microbial side, 72 Sphagnum associated heterotrophic bacteria strains, along with 12 cyanobacteria and 30 putative methanotrophs have been isolated on multiple medium types. A pilot optimization experiment confirms that our synthetic community approach is amenable to spatial characterization of target metabolites using matrix assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) along with liquid extraction surface analysis (LESA). Equipped with these resources, our team is now initiating experimentation to address the quantitative genetics of symbiosis, metabolite exchange and codependency, and ultimately how environmental perturbations interact with plant and microbial genetics to form and break symbiosis.

A Targeted Sequence Capture Approach for Resolving Flagellate Plant Phylogeny

Burleigh, Gordon

New sequencing technologies enable the possibility of generating large-scale molecular datasets for constructing the plant tree of life. As part of the NSF-funded GoFlag (Genealogy of Flagellate Plants), we used a sequence-capture approach to generate low-cost, comparable nuclear gene data that can be used to build a phylogenetic tree of all flagellate plants, including bryophytes, lycophytes, ferns, and gymnosperms. We leveraged recent transcriptome and genome sequence data to design a set of approximately 55,000 probes to amplify more than 452 nuclear exons across the flagellate plants. We describe the performance of the probes across taxonomic groups, and evaluate the performance of the probes with varying amounts of DNA and using DNA obtained from herbarium specimens. Our probe set provides a relatively simple and low cost solution to obtain the sequence data needed to construct the flagellate plant tree of life.

Phylogenomics and organellar genome evolution in bryophytes

David Bell, Steve Joya, Wesley Gerelle, Qianshi Lin, Ying Chang, Z. Nathan Taylor, Jeff D. Palmer and Sean W. Graham

We present our results of phylogenomic analyses of deep bryophyte relationships using gene sets derived from whole plastid and mitochondrial genomes. In addition to inferring deep relationships, we characterized plastid genome structural evolution, surveyed possible changes in selective regime in moss and liverwort plastid genes, and quantified differences in levels of RNA editing in the plastid and mitochondrial genomes. We performed DNA- and amino-acid based maximum likelihood (ML) analyses on separate plastid and mitochondrial alignments of gene sets derived from genomic and transcriptomic data for over 150 land plants, including 130 bryophytes, used Mauve to document major structural changes in bryophyte plastomes, and tested for shifts in selective regime by examining dN/dS ratios using PAML, with a particular focus on the mycoheterotrophic liverwort Aneura mirabilis, and a putatively mycoheterotrophic moss, Buxbaumia aphylla. We also compared transcriptomic and genomic data to investigate levels of RNA editing for a selection of species. Overall relationships among the three major bryophyte lineages and vascular plants were often inconsistent among ML analyses and weakly supported in all but the plastid nucleotide analyses. The latter consistently found liverworts sister to rest of the land plants and hornworts sister to vascular plants, with strong support. In contrast, within each bryophyte lineage our phylogenomic results were generally congruent among analyses, although there was a strongly supported conflict concerning relationships among Takakiopsida, Sphagnopsida and the rest of the mosses. An analysis of plastome structure points to extremely conserved gene order among the bryophyte lineages examined, although considerable variation in plastome size was observed, largely due to an increase in length of intergenic spacer regions in the Large Single Copy (the Inverted Repeat region of Anthoceros was also expanded in terms of gene content). We found little evidence of RNA editing among the species examined, except in the plastid genomes of Takakia and Nothoceros, and the mitochondrial genomes of Takakia, Timmia and Pallavicinia.

Ancient sex chromosome systems in plants

Carey, Sarah

Sex chromosomes have evolved several times across the tree of life. The bryophytes, whose ancestor is thought to have been dioecious, provide novel systems for understanding the evolution of ancient sex chromosomes. Given their haploid dominant nature, sex in dioecious bryophytes is determined by a UV sex chromosomal system. In this system, each sex has a non-recombining chromosome (U for females and V for males) that pair at meiosis in the monomorphic sporophyte and segregate to the male and female, haploid gametophytes. Because the sex chromosomes are transcriptionally active in the haploid stage and therefore subject to purifying selection we expect many orthologous genes will be retained between the U and V chromosomes. Here we use known sex-linked genes in bryophytes to determine the age of their sex chromosomes. We accomplish this by building gene trees, which allow us to determine not only the age of the genes on the sex chromosomes but also whether multiple capture events have occurred. Genes that are sex-linked show clear, monophyletic clusters with the known U and V-linked genes. We find that bryophyte sex chromosomes are ancient with multiple capture events of genes throughout their evolution. We additionally use these gene trees to test the of accuracy of ancestral state reconstructions of dioecy in the bryophytes.

Phylogenomics of the Funariaceae based on targeted enrichment of nuclear and organellar loci

Bernard Goffinet (University of Connecticut)
Rafael Medina (Augustana College)
Matthew G. Johnson (Texas Tech University)

The Funariaceae accommodate perhaps 200 or more annual mosses with a simple vegetative body, and a sporophyte (potentially) developing a diplolepideous opposite peristome. The diversification of the family resulted in taxa bearing sporophytes with variously reduced architectures. Phylogenetic inferences from few discrete traits resolved the deepest splits but the relationships of and within the Entosthodon and Physcomitrium complex remained poorly resolved, suggesting a rapid diversification. Inferences from all protein coding organellar loci yielded a robust phylogeny, confirming the polyphyly of two (Entosthodon and Physcomitrium) of the three most speciose genera, and consequently the nested position of several monospecific genera (e.g., Aphanorrhegma, Physcomitrella, Physcomitridium). A complementary reconstruction based on nearly 800 nuclear loci, further confirmed these phylogenetic hypotheses but also revealed the hybrid nature of several taxa, including intergeneric hybrids (see Johnson et al.), and multiple hybridizations in the Physcomitrium aggregate. The topology is congruent with the hypotheses of a) a gradual simplification of the Funariaceae sporophyte, accentuated by b) further parallel or independent reduction in various lineages. The family is at this point best considered to comprise eight genera, with the name Physcomitrella placed in synonymy with Physcomitrium.

Intergeneric allopolyploidy in Funariaceae revealed through targeted sequencing

Johnson, Matthew

Phylogenetic systematics in the Funariaceae has revealed three distinct genera: Funaria, Entosthodon, and Physcomitrium, the last of which has several distinct clades. Phylogenies reconstructed from chloroplast exome sequence place Entosthodon hungaricus within the Physcomitrium clade and suggest a hybrid origin. Using targeted sequence capture of 800 nuclear protein-coding genes, we report evidence of allopolyploid hybrids by identifying heterozygosity in gametophyte tissue. Species phylonetworks reconstructed using alleles extracted through read-backed phasing indicate E. hungaricus is an allopolyploid, a result of hybridization between an Entosthodon lineage and a Physcomitrium lineage. The analysis also reveals several Physcomitrium species, including P. immersum, are likewise hybrids between members of distinct Physcomitrium lineages. These results suggest that hybrid speciation is likely a common feature of the Entosthodon/Physcomitrium clade, and demonstrates the utility of targeted sequencing to cheaply identify allopolyploid hybrids.

The significance of whole genome duplication in history of Funariaceae

Rahmatpour, Nasim

Funaria hygrometrica and Physcomitrella patens (Funariaceae) share nearly identical vegetative bodies and differ most conspicuously in their sporophyte generation. The species occur in similar general habitats although are not ecologically sympatric. We sought to assess whether ecological and gametophytic similarities would be matched by genomic similarity or if 60 million years of divergence may be marked by ecophysiological adaptations and reflected by genomic signatures or innovations. We sequenced and characterized the transcriptome of three replicates of vegetative tissue (i.e., rhizoids, stem and leaves) of Funaria hygrometrica. We then screened the Physcomitrella genome for othologs of the Funaria transcripts to estimate their genomic divergence. Specifically, we mapped reads, transcript and protein sequences to the Physcomitrella genome. Our inferences reveal low degree of reads, transcript and protein mapping of Funaria hygrometrica to the Physcomitrella patens genome. Furhermore, in order to determine protein families of Funaria hygrometrica, its proteome were subjected to proteome of 42 model plants from PLAZA by all-vs-all BLASTp (via Orthofinder tool) as well as to proteome of three other mosses with available transcriptomic/genomic resources.
The ortho-MCL analysis reveals that 15 gene families (59 genes) are unique to Funaria hygromrtrica and 13084 genes did not assign to any gene families.
The substantial genomic divergence between Funaria and Physcomitrella was revealed in our results is in contrast with their high gametophytic similarity. The significane unsuspedted genomic innovation in Funaria might be a consequence of WGD which we could find evidence in its transcriptome. We have also investigated the effect of ploidy change (WGD) on gene expression in F1 generation of leafy gametophyte. Artifial diploid gametophyte was generated via aspospory and its gene expression was compared to normal haploid gametophyte.

Progress on Investigations of Desiccation and Diversity in Syntrichia

Fisher, Kirsten

Syntrichia is a large and diverse genus of mosses occurring worldwide and generally in dryland habitats. Despite their dominance in communities such as biological soil crusts, surprisingly little is known about the drivers of biodiversity in this clade. I will share some emerging results from an interdisciplinary project that integrates research from genomic, organismal, population, and community levels of organization to build a robust understanding of past and present dimensions of biodiversity in *Syntrichia*. The overall goal of this project is to understand the evolutionary and ecological mechanisms that have produced and maintained diversity at these different levels of organization. More specifically, the objectives are to answer the following questions: (1) What is the genomic basis for the traits that drive patterns of biodiversity in *Syntrichia*? (2) What patterns of genetic structure exist within and between populations of *Syntrichia* growing along a natural water stress gradient? (3) How does desiccation tolerance (DT) vary among and within *Syntrichia* populations in relation to sex and developmental stage? (4) How are macroevolutionary patterns and processes of lineage divergence in *Syntrichia* influenced by traits relating to reproduction and DT? and (5) What roles do phylogenetic diversity and intraspecific genetic diversity play in community resilience to environmental stress (e.g., climate change)?

Does diversity enhance resilience to climate change? A case in Syntrichia-dominated Biocrust

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In a collaborative effort to understand the dimensions of biodiversity in Syntrichia mosses, we aimed to understand the degree of resiliency to climate change in Syntrichia-dominated biocrust, and the degree to which it is attributable to diversity of the community and of the moss population. Our research is centered in the Colorado Plateau, where the genus Syntrichia is the most common biocrust bryophyte genus, and contributes to fundamental ecosystem functions and services. We explore response variables through a set of 3 studies with different climate perturbations. Our first study is a reciprocal transplant of Syntrichia caninervis and Syntrichia ruralis populations among 3 sites in an elevation-climate gradient in the Colorado Plateau, with a paired treatment of 50% shade to reduce stress. The second study also employs a reciprocal elevation transplant technique, and the same three sites, but focuses on entire transplanted biocrust communities. In our third study we monitor the effects of 8 years of a 30% reduction in precipitation on naturally occurring biocrusts, and the degree to which it is modulated by community diversity. Thus far, we have monitored changes in percent cover of Syntrichia and other biocrust community members, and will soon incorporate a desiccation tolerance assay and sex ratios. Preliminary results of study 1 show moss cover loss over a winter period without clear effects of experimental climate forcing; however, we found a positive shade effect on Syntrichia ruralis. Transplantation-induced climate warming-drying (Study 2) is altering the biocrust community, disfavoring mosses such as Syntrichia, and favoring cyanobacteria. The 30% precipitation reduction treatments of Study 3 induced a lower diversity and moss cover compared to control plots. Overall results suggest that Syntrichia-dominated biocrusts are sensitive to climate perturbation; our next steps will be to determine the influence of biodiversity on this sensitivity and to experimentally parse the influence of community species richness and genetic diversity of Syntrichia on community resilience in a new greenhouse experiment.

Summer stress signatures and simulated winter recovery: a story of hope for Syntrichia caninervis in the Mojave Desert.

Theresa A. Clark, Lloyd R. Stark, Alexander Russell, Rodolph Dagher
University of Nevada, Las Vegas

A dominant biocrust moss in the Mojave Desert, Syntrichia caninervis, faces stressful summer extremes in solar irradiance, temperature, and evaporative demand, which are predicted to increase with climate change. However, elevation, topography, and plant canopies create aridity gradients along which climatically-buffered refugia may exist for this species. Furthermore, winter/spring rain events in the Mojave create buffered hydration periods during which mosses may recover from summer stress. In order to explore these ecophysiological relationships following a summer of record-high temperatures and drought in the Sheep Mountains, Nevada, we tested the importance of source population along three nested aridity gradients (elevation, topographical aspect, and canopy shade) in predicting the photosynthetic recovery of S. caninervis under a 24hr laboratory-simulated winter hydration event (hydroperiod). The summer stress signal (chlorophyll fluorescence ratio, Fv/Fm) of S. caninervis was highly variable at 30-min post-rehydration and was strongly related to topographical exposure and canopy shade, but the nature of these patterns varied by site (3 sites spanning 1175 m elevation) and may involve putative interactions with soil drainage patterns. In 4hrs, we observed a spike in mean recovery from 0.41 to 0.72 and a 7-fold reduction in variance. Site could not explain any variation in Fv/Fm until 8hrs of recovery, at which point over 90% of recovery had occurred and site accounted for 33% of variation. By 24hrs, this percentage increased to 39-50% (via two recovery assays). Fv/Fm was strongly and positively correlated with elevation at 24hrs and this emergent site-level pattern suggests genetic signal, potentially a sign of physiological tradeoffs. Notably, all 96 samples, including those from the low-site creosote desert, reached photosynthetic performance within the range of healthy cultured specimens. These results present a hopeful story of recovery, suggesting that despite a significant spectrum in microhabitat quality and associated impacts on physiological stress, this species has the potential to recover fully from extreme summer conditions in the Mojave Desert after a modest winter hydration period of only one day.

The effects of warming on moss community dynamics in the the Western Antarctic Peninsula

Rosenstiel, Todd

The Western Antarctic Peninsula is one of the fastest warming regions on Earth, resulting in a mosaic of ice-free terrestrial habitats that are dominated by a diverse assemblage of cryptogamic plants (i.e. mosses and lichens). As the dominant terrestrial plant in this warming continent, mosses provide important habitat for a diversity of organisms yet we have remarkable little understanding of the role that moss-species or moss-functional groups play in influencing trophic level interactions in this emerging terrestrial ecosystem. Thus, it is difficult to predict how this rapidly changing landscape, driven by climate warming, will alter moss-dominated Antarctic terrestrial ecosystems. Here, we used Open Top Chambers (OTCs) on King George Island, Antarctica, to examine the effects of passive warming and moss species on the abiotic environment and ultimately on higher trophic levels. In moss-dominated ecosystems where two moss species, Polytrichastrum alpinum and Sanionia uncinata make up over 65% of the vegetative cover, we found strong species-specific effects on the abiotic environment in moss canopy temperature and soil moisture. We found distinct reproductive shifts in P. alpinum under passive warming compared to those without warming, and invertebrate communities in this moss species were strongly correlated with reproduction. Moss communities under warming had substantially larger total invertebrate communities, and these invertebrate communities were influenced differentially by the moss species for some taxa but not others. However, warmed moss systems showed lower fungal biomass than those in control moss communities, and fungal biomass differed between moss species. Our results suggest that continued warming will differentially impact the reproductive output of Antarctic moss species and is likely to alter terrestrial ecosystems dynamics, including invertebrate communities, from the bottom up. Understanding these effects requires clarifying the foundational, mechanistic role that individual moss species play in mediating complex interactions in Antarctica's terrestrial food-webs.

Student Achievement in a Fully Online Plant Diversity Course: Which Instructional Components are Better to Use?

Dogan, Selcuk

The trend and demand for online education has gradually grown nationwide. Increasing number of universities are now offering fully online programs with the focus on science courses. University of Florida (UF) Online is one of those programs that has offered bachelors' degrees in Biology. One of the courses offered is a 2000-level undergraduate Botany lab course, Plant Diversity, which includes innovative instructional components, such as labs, peer-reviewed discussions, and active learning, collaborative opportunities. This study sought to examine the extent to which these instructional components explain student learning in a structured online environment. Based on the data collected in the beginning, during and at the end of the course through surveys and tests from 108 students, we created a regression-based model. The results showed that labs and discussion-based activities affected student learning positively, controlling for their pre-knowledge in the beginning of the course. This finding informs the current practices in the course and other online biology courses at UF or other institutions. The evidence helps instructors and instructional designers when creating a fully online course that takes academic failure into account.

Cyberlearning Activities on Flagellate Plants to Improve Learners' Knowledge of Flagellate Botany and Perceptions of Science Learning

Valle, Natercia

This study is part of a NSF-funded project that focuses on phylogeny of flagellate plants and educational research and development to increase awareness and appreciation of flagellate plants and their crucial role in our environment. The 5E Instructional Design Model (engage, explore, explain, elaborate, and evaluate) was the instructional design approach used to develop cyberlearning activities around socially relevant themes such as the role of lycophytes in coal production and the world carbon bank. The real-world connections of flagellate plants to many topical issues in our society, such as energy production, are significant but not obvious to a layperson or a novice student in biology programs. Specifically, we piloted 3 innovative cyberlearning activities on the role of flagellate plants in an online undergraduate Botany course during the Spring of 2018 semester with 105 enrolled students (74 female, mostly white, in junior and senior year). The Opinions About Science and Science Learning sub scale from well-known Classroom Undergraduate Research Experience (CURE) survey was used to assess learners' perceptions of the cyberlearning activities in relation to their opinions about science and science learning. An overwhelming majority of students liked or strongly liked the 3 modules and thought they were useful and user-friendly. More nuanced qualitative and quantitative data will be reported during the presentation.

Building a platform for authentic undergraduate research experiences using the sexually dimorphic moss Ceratodon purpureus

McDaniel, Stuart

An authentic undergraduate research experience is a powerful educational tool that can generate excitement for science, improve learning outcomes, and promote retention in STEM fields. Here we describe the development of laboratory platform accompanied by a multi-dimensional Ceratodon purpureus data set that will serve as a foundation for discovery-based undergraduate research. The moss C. purpureus is well-suited for undergraduate research because laboratory isolates are easy to clonally propagate, cultivate under a variety of conditions, and share among labs. We piloted this project first with undergraduates in a research lab setting and second as a multi-week laboratory exercise in Introductory Botany at the University of Florida. Undergraduates demonstrated learning of valuable, translatable laboratory skills, including sterile tissue culture, fitness assays, growth tests, image analysis, DNA extraction, PCR-RFLP, and gel electrophoresis, as well as conceptual skills related to experimental design, hypothesis testing, statistical analyses, and data presentation. The next steps in this project are first to incorporate existing phenotypic, transcriptomic, and metabolomic data into the introductory scaffolding to provide students with the opportunity to generate and test increasingly sophisticated biological hypotheses, and second test the activities in other student populations.

Overlooked diversity in Epipterygium

Hanusch, Max

In the nineteenth and early twentieth centuries, bryophyte species were seen as organisms with small distribution ranges and low variability. This led to the assumption that vicariance and geographic isolation play a major role in bryophyte speciation. Later, when intraspecific morphological variation, long distance dispersal and low rates of molecular evolution in bryophytes were accepted among bryologists, a great number of local species were synonymized. As a result, many bryophyte species now show broad transcontinental geographic ranges, often with multiple disjunct distribution areas. Epipterygium tozeri (Bryaceae) is such a case, showing a holarctic distribution with disjunct areas in the western parts of North America, the Mediterranean and central Asia. Specimens from different geographic regions were first described as independent species and later, many were lumped in E. tozeri based on morphology. Here we contrast the morphological species concept in the genus Epipterygium with molecular data. A combined analysis of one nuclear and two plastid loci was performed to assess species circumscriptions and distribution patterns and to reconstruct the historical biogeography in the Epipterygium tozeri complex.

Elucidating the role of N-acylethanolamine mediated signaling pathway in Physcomitrella patens

Imdadul Haq and Aruna Kilaru

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In plants, saturated and unsaturated N-acylethanolamines (NAEs) with acyl chains 12C to 20C are reported for their differential levels in various tissues and species. While NAEs were shown to play a vital role in mammalian neurological and physiological functions, their metabolism and functional implications in plants however, remain incomplete. Fatty acid amide hydrolase (FAAH) is one of the metabolic enzymes that break the amide bond in NAEs to release free fatty acid and ethanolamine. FAAH orthologs, putative PpFAAHs (Physcomitrella patens FAAH) were identified based on the sequence blast of ratFAAH and named as PpFAAH1 to PpFAAH9. Based on the highest mRNA expression of the PpFAAH homologs upon NAE treatment, PpFAAH1 was selected for further in vitro characterization, which share 31% sequence identity with ratFAAH. PpFAAH1 was heterologously expressed in E. coli and purified for characterization. Highest amidohydrolysis activity of PpFAAH1 was observed in vitro at pH 8.0 and temperature 37°C. Methoxy arachidonyl fluorophosphonate (MAFP), an inhibitor showed highest inhibition with 10mM concentration, however, one of the principal classes of FAAH inhibitor O-aryl carbamates (URB597) exhibited only 22% inhibition with the same concentration. Both in vivo and in vitro studies showed that unsaturated NAE substrate (NAE 20:4) is hydrolyzed faster than the saturated NAE (NAE16:0); more than 50- and 10-fold higher in vitro and in vivo assays, respectively. Amidohydrolase activity in vivo was mostly associated with microsomes compared with cytoplasmic fractions. Additionally, microsome fraction of mature gametophytes showed higher amidohydrolase activity than of the protonemal or early gametophyte stages; however, PpFAAH expression was not significantly different between the developmental stages. Further functional characterization of NAE metabolic pathway is ongoing by generation of PpFAAH knock out (KO) and overexpressor (OE) to understand the biological implications of FAAH in growth and development of early land plants.

Biochemical Characterization of SABATH Methyltransferases in Liverworts

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The SABATH family is a mid-sized gene family that encodes enzymes catalyzing the methylation of small molecule carboxylic acids in plants. Some substrates of the SABATH enzymes are phytohormones that include indole-3-acetic acid, gibberellic acids, salicylic acid and jasmonic acid and others are considered secondary metabolites. While the SABATH methyltransferases have been well studied in a number of seed plants, our knowledge of them in nonseed plants is very limited. Among nonseed plants, liverworts are considered to be most related to ancestral land plants. As such, the information about the SABATH family in liverworts may provide new insights into the origin and substrate specificity evolution of this group of enzymes. In this study, comparative genomic and biochemical methods were applied to study the SABATH enzymes into liverworts, Marchantia polymorpha as the only liverwort with genome sequenced and Conocephalum salebrosum as a liverwort with great scent. In M. polymorpha, three SABATH proteins that have activity with jasmonic acid were identified. In C. salebrosum, one SABATH that methylates cinnamic acid and one methylates salicylic acid were identified. Phylogenetic analysis of these proteins with their counterparts in seed plants provided new insight into the functional evolution of the SABATH family.

Evolution of Cellulose Synthase Functional Specialization

Alison W. Roberts, presenter
Xingxing Li, Mai L. Tran, Arielle M. Chaves, Joanna H. Norris

Based on phylogenetic analysis, the common ancestor of seed plants and mosses had a single CELLULOSE SYNTHASE (CESA) gene and thus homo-oligomeric cellulose synthase complexes (CSCs) composed of identical subunits. Seed plants use different CESA isoforms for primary and secondary cell wall deposition and have obligate hetero-oligomeric CSCs that assemble and function in planta only when all three required CESA isoforms are present. Our investigation of the independent diversification and functional specialization of the CESA family in the moss Physcomitrella patens sheds light on the selective pressures to drove the evolution of specialized primary and secondary cell wall CESAs and hetero-oligomeric CSCs. Like seed plants, P. patens uses different CESA isoforms to synthesize primary cell walls (PpCESA5) and secondary cell walls (PpCESA3 and PpCESA8). This is consistent with convergent evolution of secondary cell walls with aggregated and helically oriented microfibrils, enabled by regulatory uncoupling of primary and secondary cell wall synthesis through CESA subfunctionalization. PpCESA3 and PpCESA8 form obligate hetero-oligomeric complexes with B-clade PpCESA (PpCESA4, PpCESA6, PpCESA7 or PpCESA10). This is consistent with convergent neofunctionalization of CESA-CESA interfaces, as predicted by the Constructive Neutral Evolution hypothesis. In contrast to PpCESA3 and PpCESA8, PpCESA5 does not interact with other PpCESA isoforms. Thus, P. patens has both homo-oligomeric and hetero-oligomeric CSCs. The ability to compare CESAs that form homo- vs. hetero-oligomeric complexes, along with the high sequence identity between PpCESA isoforms, may facilitate identification of specific residues responsible for neofunctionalization of CESA-CESA interfaces. This work was supported by National Science Foundation Award IOS-1257047.

Behavior of SMC5/6 complex in the moss Physcomitrella patens

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SMC5/6 is an essential complex, which is involved in the range of vital cell pathways including repair of double-strand breaks of DNA (DSB) via homologous recombination. Because knock-out mutants of smc5 and 6 are not viable in the moss Physcomitrella patens similarly as in other organisms from bacteria and yeast to men we used RNAi silencing to study impact of interference with transcription on phenotype. To silence SMC6 we constructed RNAi vector pKA228 containing inverted repeats of 600 nucleotides long fragment of SMC6 cDNA driven by in moss strong ubiquitin promoter. Hairpin RNA transcribed from the pKA228 targets 5'- end of SMC6 mRNA coding ATPase head of protein. Modification of SMC6 expression causes restricted development of leafy gametophyte and specific "annular ring-like" phenotype of transformants with necrotic tissue in the middle and fresh growing tissue around the perimeter.
As expected, in the first subculture starting from primary transformant the level of SMC6 transcript is reduced and according to similar phenotype we picked three stable lines 228-5, 6 and 7 for detailed studies. Nevertheless, during further propagation with weekly subculture, all three lines revealed dependence of SMC6 transcript-levels on the number of performed subcultures. When compared to wild-type, the level of SMC6 transcript is reduced during the first three subcultures and afterwards starts gradually increase up to two-fold level higher of the wild-type at subculture 6. The changes in SMC6 transcript-levels are accompanied by changes in transcript-levels of other members of SMC5/6 complex. Particularly pronounced is increase of SMC5, NSE1, NSE2 and NSE4 transcripts, whereas level of NSE3 transcript seems to be rather decreased at the time, when SMC6 transcript is reduced. The upregulation of the non-SMC NSEx components can be response to lack of functional SMC5/6 complex backbone. Nevertheless, sharp almost three-fold increase of NSE1 and NSE2 might be related to their ubiquitin/SUMO ligase activity. The E3 ligases might be involved in deactivation of defect/insufficient pathway by proteolysis some of its proteins and thus free up the space for proteins of alternative pathways.
Changing levels of SMC6 transcripts in smc6-5,6,7 mutants during continuous propagation are associated with different sensitivity/resistance to model genotoxins tested by spot-inocula phenotype assay and DSB repair rate assessed by comet assay. RNAi interference with SMC6 has also dramatic effect on spontaneous as well as Bleomycin induced APT- mutagenesis

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Fast, fat and fragrant moss

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The moss Physcomitrella patens is now established a fragrance producer. Currently, the fragrant mosses are sold as living plants to consumer in US (www.orbellamoss.com), and further work are ongoing to use it for fragrance production. However, this lovely little plant do not grow as fast as yeast, and the fragrant compounds are likely to leave the cell during production.

In order to address these problems we engineered P. patens to grow faster (at least 20%), produce more lipid droplets and enable a faster and higher yielding production of fragrances. We showed that P. patens had an increased rate of growth at least 20% based on dry biomass measurements as compared to the WT. This was obtained by overexpression of cyclins and cyclin dependent kinases.

We also showed that we could increase the numbers of lipid droplets made in the cell, from a few to a cell packed with lipid droplets. This was obtained by overexpression of lipid droplet associated proteins, which is in line with work in other plants. We observed that fragrance produced in cell with high lipid droplet numbers harbour a larger amount fragrance in the cell verses in the medium as compared to cell without extra lipid droplets. Thus, more fragrance is trapped and can extracted at a later stage. This work also showed that overexpression of lipid droplet associated proteins could increase the growth of the cells. Thus, one could obtain more cell biomass containing more fragrance in the cells. Clearly a benefit for any future use of P. patens as a small molecule producer.

In order to obtain these different fragrant mosses we also engineered novel promoters, used different terminators and linkers. This will also be addressed, and show that it is important to continuously to increase the number of synthetic biology parts that can be used in mosses. We designed three short synthetic promoters and characterized them by the use of the fluorescent protein VENUS. All of the tested promoters were active, and showed activity higher than the than the frequently used 35S. The study show that few cis-elements is enough to establish a strong promoter for continuous expression of genes in plants.

Overall, we have established a fast and fat P. patens line and increased the number of test synthetic biology tools. In the just started MossTech project (www.mosstech.eu) we use these results and expand the use of bryophytes within synthetic biology and provide novel biotechnological solutions for the green biotech industry.

Investigation of various extraction methods for isolation of bioactive components of Dryopteris erythrosora

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Dryopteris erythrosora, a fern native to China and historically used in traditional Chinese medicines, was chosen to compare the effectiveness of nine different extraction techniques and analyzed for its bioactive properties and compounds. The extracts were then evaluated by both the Total Phenolic Content (TPC) assay and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay to analyze antioxidant properties. Extracts were also assessed by the Kirby Bauer disc diffusion method to view antibacterial properties. All extracts were analyzed with reverse phase HPLC/ESI-MS and GC-MS to compare the difference in phenolic compounds extracted between techniques. The extract obtained by a deep eutectic solvent (DES) synthesized from a 1:2 ratio of choline chloride:ethylene glycol, diluted in 25% H_2O, exhibited the greatest antioxidant activity. No antibacterial properties were detected within the extracts. Therefore, D. erythrosora was confirmed to show antioxidant capabilities, however, it did not display antibacterial activity. Future work involves using standards of different phenolic compounds to compare and confirm the contents of each extract.

Investigating Phosphatidylinositol 4-phosphate function in Physcomitrella patens by CRISPR/Cas9 mutagenesis of Type III Phosphatidylinositol 4-kinase genes

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Phosphoinositides (PIs) are key signaling lipids involved in many fundamental cellular processes. In Arabidopsis, the PI, PtdIns4P, is involved in polarized growth of root hairs and pollen tubes, chloroplast movements and division, modulation of stomatal opening/closing and salicylic acid-dependent immunity. In contrast, there is limited work on PtdIns4P in non-vascular plants. We are using reverse genetics to dissect the function of PtdIns4P in Physcomitrella; specifically we are using CRISPR/Cas9 mutagenesis to knockout Type III Phosphatidylinositol 4-kinases (PI4Ks) that produce Ptdins4P. Physcomitrella contains four Type III PI4K genes, PI4Ka1, PI4Ka2, PI4Kb1 and PI4Kb2. We report the initial characterization of pi4ka1 and pi4ka2 mutants. Multiplex CRISPR/Cas9 editing yielded only single mutants, suggesting that pi4ka1/a2 double mutants are lethal. Frame-shift mutants, predicted to yield truncated proteins, were identified as pi4ka1 and pi4ka2 null mutants. Polarized cell growth was unaffected in both pi4ka1 and pi4ka2 mutants; there was no difference from wild-type, in size or solidity of plants regenerated from protoplasts or in the size of colonies grown from protonemal cell suspensions. However, the number of leafy gametophores produced by pi4ka1 mutants was half that observed in pi4ka2 or wild-type colonies and the pi4ka1 gametophores were abnormal in morphology with irregularly shaped phyllids, composed of disorganized, misshaped cells. These results point to a novel role for Physcomitrella PI4ka1 in 3-dimensional growth and patterning of cell divisions during phyllid development.

The Effect of Growth on Competition Between Sexes of a Dioecious Bryophyte

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Sex ratio bias is commonly observed in dioecious plants. More focus has gone into studying the phenomenon in Angiosperms, and less among flagellate plants, such as Bryophytes. In species documented showing sexually dimorphic traits, displacement of one sex by the other can occur if a dimorphic trait gives one sex higher competitive ability than the other. Sexually dimorphic traits such as desiccation tolerance, germination percentage, asexual reproduction, and growth rates have been documented to explain sex ratio bias. However, in Bryophytes competition between the sexes has not been tested and a dimorphic trait has not been experimentally linked to a difference in competitive ability. This study measures competition between the sexes and tests for the impact of growth rate difference on the competitive effect of one sex on the other. Plants of known growth rates were used to create three growth rate combination groups; high growth rate females with low growth rate males, medium growth rate females with medium growth rate males, and low growth rate females with high growth rate males. Final sex ratios favoring higher growth rate groups will indicate that growth rate difference was a factor determining final sex ratios. Currently, after the first two months, there are no significant differences in relative growth rate between either growth rate groups or between sexes. However, initial size of thalli cutting is having a significantly positive relationship to relative growth rates. This effect is expected to be less influential as the experiment progresses and plants begin to physically interact. If sex ratios in Bryophytes are strongly determined by competitive interactions between the sexes, then differences in competitive ability can predict biased sex ratios. If the degree of dimorphism in growth rate determines the competitive effect of one sex on the other then dimorphic growth rates can lead to biased sex ratios.

Biosynthesis of Terpenoids in Marchantia polymorpha

Wei, Guo

Terpenoids constitute the largest class secondary metabolites in land plants. Compared to the rich knowledge in seed plants, little is known about terpenoid biosynthesis in nonseed plants. It was recently reported that nonseed plants contain microbial type terpene synthase genes (MTPSL) and typical plant terpene synthase genes (TPS). The model liverwort Marchantia polymorpha produces a diversity of terpenoids. This study investigates the molecular and biochemical basis that determines terpene diversity in M. polymorpha. From its sequenced genome, 32 MTPSL genes and 7 TPS genes were identified. In addition, 5 genes encoding trans-isoprenyl diphosphate synthases, which produce substrate for terpene synthases, were identified. Through metabolites-gene or gene-gene coexpression analysis, candidate terpene synthase genes and IDS genes were identified. Their catalytic activities were determined using in vitro enzyme assays.

UV tolerance in Mojave Desert biological soil crust mosses

Jenna Baughman

As poikilohydric organisms, terrestrial mosses will dehydrate and go dormant between precipitation events. Although many mosses are found in cool, low light environments, several species are abundant in drylands. This research investigates ultraviolet (UV) protection mechanisms used by the desert mosses Syntrichia caninervis and S. ruralis. These species are highly desiccation tolerant; they can lose almost all of their cellular water and recover after rehydration. In nature, desert mosses not only have to withstand the damage of desiccation itself but must also be able to recover from any damage incurred while inert or have adequate mechanisms for injury prevention. Mosses have no ability for active repair when dry and face risk of damage to sensitive molecules, including DNA, which absorbs wavelengths in the UV spectrum. Desert Syntrichia species develop a dark brown coloration in nature, a plastic trait that does not occur in low light conditions and that may provide protection from solar radiation. To investigate UV protection mechanisms used by S. caninervis and S. ruralis, clonal replicate cultures were exposed to a pulse of UV-B radiation and their transcriptomes were compared for differential expression and candidate genes for UV tolerance. Simultaneously, clonal replicates were grown in a broad-spectrum UV and visible light environment in a growth chamber to test the long-term effects of high and low UV radiation. Liquid chromatography mass spectroscopy was used to characterize metabolites that are differentially produced and that absorb wavelengths in the UV spectrum. Finally, in a paired design, 40 patches of S. caninervis were covered with either a UV-filtering (>80% reduction) or UV-transmitting (>80% transmission) window, 5 in.
x 5 in. After 1 year, branches from each pair, as well as from a third, equivalent but unmodified microhabitat, will be collected and compared for key metabolites and transcripts. The transcriptomic and metabolomic analyses from controlled experimental and modified natural conditions will uncover detailed information about how these species respond to UV and desiccation, contributing to our understanding how they survive the extreme conditions of deserts and drylands.

Development of new genetic resources for Sphagnum fallax

Adam L. Healey, John T. Lovell, Chris Plott, Jerry Jenkins, Jane Grimwood, Shu Shengqiang, Kerrie Barry, Jonathan Shaw, David Hanson, David Weston, Jeremy Schmutz

Approximately one third of all organic carbon is currently stored in northern peatlands, where CO2 deposition is greater than release due to peat's recalcitrance to microbial degradation. This carbon balance is mediated by plant and microbial ecological communities within peatlands, of which Sphagnum represents an important taxon. However, despite its role in global carbon cycling, Sphagnum genetic resources are lacking both a chromosome level genome assembly and a high-quality genetic map. To remedy this, Sphagnum fallax was sequenced to a depth of 93X with PacBio long-read sequencing and assembled using MECAT. The resulting genome assembly was 412.2 Mb in 226 contigs and a contig N50 of 17.5Mb, representing ~140X improvement in contiguity over the current S.fallax genome (v0.5). In order to generate a high-density genetic map, a fallax-fallax F1 cross was generated from 186 individuals and sequenced to a depth of 15X using Illumina 2x150bp PE reads. Each library was then aligned to the MECAT assembly using BWA and calling SNPs using VARSCAN. This pipeline generated a matrix of 2.5 million SNP markers, of which 1.1 million could be phased using on the maternal parent of the cross. After culling markers with high LD, low recombination frequency and those within < 1 cM of each other, the final genetic map was a total length of 5395.77 cM, composed of 2,990 markers with an average spacing of 1.8 cM. To generate chromosomes, 1.08 million SNP markers that were correlated with the final genetic map (r >0.95) were mapped back to the MECAT contigs in order to assess misjoins in the assembly and define linkage groups. After correcting misjoins and identifying telomeric sequences, contigs were joined into 19 linkage groups, representing 399 Mb (95% of contig sequence). The newly generated genetic map and chromosome assembly of S. fallax will aid ecological studies of Sphagnum, QTL gene discovery and enable functional genomic studies among moss species and land plants.

Automated Method of Image Analysis Using ImageJ Macro Programming Language

Khanal, Tikahari

Image analysis is often utilized in scientific research to quantify a broad range of image details. High throughput methods of image analysis allow for more images to be analyzed in a shorter period of time without decreasing accuracy and precision. Despite the relative abundance of automatable steps present in many procedures of image analysis and the ability of algorithms to account for subjective observation, high throughput methods of image analysis remain underused across many research facilities. In a study investigating sexually dimorphic traits, automated methods of image analysis were implemented in order to analyze more pictures using a standardized protocols. To account for variation in image content and integrate subjective user input, two solutions were applied: images were either analyzed by a high-throughput algorithm and classified as likely mistakes or correct images, or they were analyzed individually using an algorithm that accounted for user input. The algorithm corresponding to area and perimeter measurements made use of the difference in hue between the leaf and its corresponding background and the algorithm corresponding to length measurement automated the procedure of data collection to the point of limiting user input to a single line rendering. Protocol of user input and interaction with the automated leaf length data collection procedure was determined through trials implementing the different line drawing tools available on ImageJ software while the protocol for the automated leaf area and perimeter data collection procedure involved a third macro that allowed for the successive viewing and possible recollection of data of images labelled as mistakes. Almost all forms of software-based image analysis can be automated to a point and be broken down into high-throughput procedures similar to the one developed in this experiment. Application of the principles behind this method of image analysis in the development of these automated methods of image analysis can significantly increase the quantity of data without affecting quality and could benefit many research facilities.

Balancing selection in a deletion polymorphism in the moss species Ceratodon purpureus.

McBreen, Jordan

A major goal in evolutionary biology is to understand how natural populations maintain genetic variation through a number of selective forces; one selective force of interest to the lab is balancing selection. Balancing selection is a phenomenon in which multiple alleles of a given gene are maintained within the gene pool in order to increase genetic variation. In this research, we studied a deleterious phytochrome gene (CpPHY1) deletion in the moss Ceratodon purpureus. Deletion individuals have decreased fitness in that they produce less germinating spores than individuals with CpPHY1. Here, we aimed to determine the selective forces behind the maintenance of this novel gene polymorphism.

The flanking regions of the PHY1 gene in both PHY1 present and absent individuals were sequenced, resulting in amplicons of DNA that could be compared and used in classic population genetic analyses. Data from Sanger sequencing showed increased nucleotide diversity in the regions flanking the PHY1 gene, a hallmark of balancing selection. This data led us to determine that balancing selection could be responsible for maintaining the novel gene polymorphism in populations of Ceratodon purpureus. Further analyses and studies can be carried out to confirm that balancing selection is in fact the culprit as to how a gene polymorphism that decreases individual fitness is being maintained within and across gene pools. We must investigate why it is being maintained. Further research will involve evaluating fitness in CpPHY1 present and deletion individuals.

Exogenous glucose alters the metabolism in the moss Physcomitrella patens independent of its osmotic effect

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Both microorganisms and multicellular organisms coordinate their metabolic activity according to changes in nutrient availability. In plants, sensing the availability of energy in the form of sugars is particularly critical since these molecules play a key role in different processes that impact their growth and development. To know the total effect of P. patens tissues exposed to high sugar concentration (glucose), we performed mass spectrometry fingerprinting with DIESI-MS. Also, to explore some of the biochemical adjustments caused by the treatments, levels of sugars such as glucose, fructose, sucrose and starch were also determined. Additionally the photosynthetic efficiency, chlorophyll and carotenoids content of P. patens were measured. The results showed that the fingerprint of Glucose feeding was specific and different from an osmotic effect. Moreover, glucose treatment led to a strong increase of both glucose and fructose, while sucrose and starch content decreased. Surprisingly, these changes did not affect the photosynthetic efficiency. Feeding with an osmotic agent (sorbitol) caused a marginal decrease of internal glucose levels but did not alter the pools of fructose, sucrose and starch neither photosynthetic efficiency compared to control conditions. In conclusion, the ionomic approach and the metabolic survey of sugars confirm that glucose causes a specific response that is independent from the osmotic effect. Moreover, P. patens possesses a higher tolerance to osmotic and glucose-induced photosynthetic inhibition compared to vascular plants, highlighting important differences between these two group of plants in response to both stimuli. We thank INSTITUTO POLITÉCNICO NACIONAL, SECRETARIA DE INVESTIGACION Y POSGRADO, SIP 2018, COFAA and CONACyT for financial support.

Insights into the proteomic response to high glucose levels in the moss Physcomitrella patens

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Sugar levels in plants are fine-tuned according to the plant development stage and environmental factors through different signaling pathways. Particularly, glucose signaling has been widely studied in the vascular plant Arabidopsis thaliana, but it has remained largely unexplored in non-vascular plants, such as Physcomitrella patens. Thus, we decided to explore the proteomic response of P. patens to high glucose levels. Total protein extracts from glucose treated protonemal tissues were analysed by label-free LC-MS. Our analysis revealed that high glucose levels are sensed and induce proteins mainly related to oxidative stress, carbohydrate metabolism, photosynthesis, and cytoskeleton in P. patens. According to the proteins identified, such glucose-derived oxidative stress is countered mainly by the ascorbate-glutathione cycle, but also other mechanisms like the pentose phosphate pathway and non-photochemical quenching processes. In response to sorbitol (osmotic control) up-regulated proteins involved in translation, signaling, ATP synthesis and carbohydrate metabolism, were identified. Interestingly, only one protein was identified as common to glucose and sorbitol treatments. According to these results, our study reveled that high levels of glucose evoke an oxidative stress response in P. paten something that has not been reported in A. thaliana, and this response is specific and independent from the osmotic effect. We thank INSTITUTO POLITÉCNICO NACIONAL, SECRETARIA DE INVESTIGACION Y POSGRADO, SIP 2018, COFAA and CONACyT for financial support.