

Phylodiverse and large-scale data perspectives on the origin of land plants

Project TerreStriAL is studying algae in a bid to understand how land plants evolved. By analysing the genes and functions of these algae, scientists are uncovering the genetic secrets and adaptations that allowed plants to thrive on land.

Our planet's surface is covered by macroscopic photosynthesising organisms: the land plants. Land plants make up the majority of the biomass on land and are extremely diverse. On a stroll through the forest, one might appreciate the diversity of land plants ranging from mosses and liverworts that cover rocks to the trees surrounding one. They are the crops that cover our fields. They are the cacti that add spots of green to a desert. All of these organisms can be traced back to a single common ancestor, whose descendants give rise to the major lineages of land plants. But where did this ancestor come from?

Streptophyte algae: key players in understanding plant conquests

Land plants belong to a larger group of photosynthetic organisms called streptophytes. Albeit being the most species-rich group in the streptophytes, streptophytes include more than just land plants. They also feature a range of freshwater and terrestrial algae called the streptophyte algae. Both land plants and streptophyte algae are essential to our understanding of the conquests of land by plants. While there is a wealth of data on land plants, we are just beginning to delve into the molecular biology of streptophyte algae. This is what our work is all about.

In TerreStriAL, a European Research Council-funded project (European Union's Horizon 2020 research and innovation programme), we take the biodiversity of land plants and their conquest of diverse habitats into account to understand the unique success of land plants. Since land plants can dwell in diverse and challenging environments, the dynamic molecular toolkit that land plants use to respond to adverse environmental conditions is likely a basis for their success. We ask the question of which genetic components were present in the earliest land plants. For this, we take a comparative and complementing approach that builds on diverse methods. The main pillars are experimental work and comparative genomics on the algal relatives of land plants, the streptophyte algae.

Comparative analyses with the aim of inferring evolutionary processes hinge on having a robust backbone of the relationship between the groups of interest: a phylogeny. In my lab, we work with organisms that are alive today—a snapshot in time and biodiversity. To trace evolutionary events of the distant past, we turn to the genetic information contained within a diverse set of extant species and use these data to trace their shared ancestry. In practice, this means extracting the nucleic acids that store the genetic information and sequence it. By comparing the sequences of shared genes, we can then deduce the degree of changes that occurred during their evolutionary history—a degree that correlates with the time of divergence. Nowadays, the gold standard is to build such molecular phylogenies not based on one or a few genes but based on many hundreds or even thousands. This is called phylogenomics. We recently applied phylogenomics to important groups of streptophyte algae salient to our understanding of the deep evolution of land plants.

In 2021, we published a study in *Proceedings of the Royal Society B* (Irisarri *et al.*, 2021), where we investigated the phylogenetic relationship of one of the streptophyte algal groups most distant to land plants, the Chlorokybophyceae. The Chlorokybophyceae, along with another class of streptophyte algae called the Mesostigmatophyceae, form a clade of streptophytes that diverged from land plants one billion years ago. Both of these classes were thought to consist of only one species each. We scrutinised the relationship within Chlorokybophyceae by sampling diverse strains of *Chlorokybus*, extracting and sequencing their nucleic acids, using phylogenomic analyses based on a matrix that contained 529 genetic loci sampled from tens of thousands of candidate loci. Using these loci for phylogenomic analyses revealed a surprise.

Though indiscernible through their appearance, these different strains fell into the class of Chlorokybophyceae with a deep genetic structure; this spoke of different species. We, therefore, had

uncovered an unbeknownst (cryptic) species complex. Studying this diversity at 'the other end' (relative to land plants) of the streptophyte tree of life will be important for our understanding of trait evolution in streptophytes.

Unveiling the Secrets of Closest Plant Relatives: Zygnematophyceae

We also turned our phylogenomic eye to the algal class most closely related to land plants: the Zygnematophyceae. This study was carried out together with many colleagues, especially from Hamburg and Cologne, and was published in 2022 in *Current Biology* (Hess *et al.*, 2022). Here, we included an enigmatic organism that was described more than 100 years ago but, since then, had almost been forgotten, *Mougeotiopsis*.

Mougeotiopsis is a filamentous zygnematophyte that lacks one of the usually common features of green algae: a pyrenoid. Pyrenoids have a sub-cellular structure (visible as dark green spots in light micrographs of algae) that allows for efficient carbon fixation through the dark reaction of photosynthesis. This alone made *Mougeotiopsis* highly interesting. Thus, we included this peculiar organism in our phylogenomic survey of 46 Zygnematophyceae.

We again used a matrix of hundreds of shared genes (326 in this case) and computed a robust phylogeny. This revealed an interesting pattern: Zygnematophyceae with a multicellular body plan were scattered over the three. This means that the trait of multicellular growth was either gained or lost multiple times throughout the evolution of land plants' closest algal relatives. This is important, as multicellular growth is what allows for the complex bodies of land plants.

To scrutinise the evolutionary history of multicellularity in zygnematophytes, we applied a method called ancestral character state reconstruction. This revealed that multicellular growth was likely present in the last common ancestor of algae and land plants but not present

in the last common ancestor of land plants' closest algal relatives, where it was gained multiple times independently. As illustrated by the case of the evolution of multicellularity, the closest algal relatives of land plants, the Zygnematophyceae, carry essential information for our understanding of the biology of the earliest land plants. We have recently performed two major functional genomic investigations on Zygnematophyceae.

On the unicellular system *Mesotaenium endlicherianum* (Figure 1), a freshwater algal member of the Zygnematophyceae, we used a 2D gradient table to examine how variations in temperature and irradiance affected the alga's gross physiology and combined this with functional genomic analyses. The results have been posted on the pre-print server for biology, *bioRxiv* (Dadras *et al.*, 2022).

To have a proxy for the physiological status of the algae, we worked with the maximum quantum yield of photosystem II. Our study revealed that the algae's growth and physiology declined with rising irradiance levels, with the lowest photosystem performance values observed under conditions of high irradiance and low temperature.

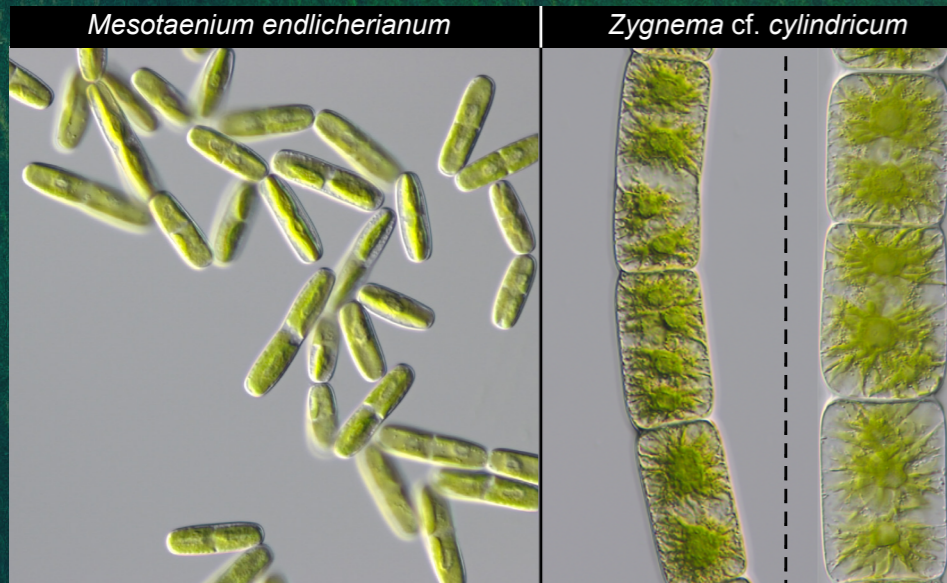


Figure 1: Two zygmatophyte systems used by the de Vries lab for functional genomics, the unicellular alga *Mesotaenium endlicherianum* SAG 12.97 and the filamentous alga *Zygnema cf. cylindricum* SAG 698-1a.

In addition to physiological analyses, we performed global gene expression analyses using RNAseq to identify the molecular mechanisms that underpin the alga's responses to environmental stressors. We extracted RNA from more than a hundred samples and sequenced a total of 1.5 trillion bases. We used these data to update the *Mesotaenium* gene models, which almost doubled the number of known protein-coding genes for this organism. The new gene models will be an important open-access resource for the entire community.

Unravelling gene expression in algae's response to environmental stress

We next moved on to identify potential molecular mechanisms underlying the alga's responses to environmental stressors using a diverse set of approaches. For this, we made use of the gene expression responses of *Mesotaenium endlicherianum*, to the environmental cues of light intensity and temperature—some of which elicited palpable physiological stress. Differentially expressed genes by comparing gene expression levels between different groups shed light on the biological pathways associated with these genes. Especially genes associated with the biology of the chloroplast were frequently enriched among the most differentially regulated genes.

To further investigate how gene expression is coordinated in response to different environmental cues, we used weighted gene co-expression network analysis to cluster genes into modules and identified hub genes that are highly connected in the gene network. Many of the clusters were associated with plastid biology and physiology, and we identified conserved hub genes for plastid-derived signals.

Overall, our study provides insights into the genetic programmes that are activated in *Mesotaenium endlicherianum*

in response to environmental cues and highlights the importance of plastid biology in this alga's overall cell biology. Our approach of using network analyses to identify hub genes that are highly connected in the gene network is a powerful tool for understanding how gene expression is coordinated in response to different environmental cues and shared across land plants and their algal relatives.

In a joint international team effort, spearheaded by Prof. Yanbin Yin and us, we have assembled chromosome-scale nuclear genomes of four strains of the filamentous zygmatophyte *Zygnema*. The results have been posted on the pre-print server for biology, *bioRxiv* (Feng *et al.*, 2023). For this, we used a powerful combination of: (i) Illumina short read data, (ii) modern long read data generated by PacBio High-Fidelity (HiFi) and Oxford Nanopore; and (iii) chromatin conformation data to scaffold the genome, resulting in 20 pseudo-chromosomes. The nuclear genome

assembly of one strain was four times larger than those of the other strains, suggesting that it is a diploid organism with a high heterozygosity rate. Our study provides the first chromosome-level assemblies for any streptophyte alga, which will be a great open-access resource for everyone in plant science.

We next explored functional gene modules in *Zygnema circumcarinatum* by inferring gene co-expression networks from RNA-seq data. We obtained more than 400 cohorts of co-expressed genes that allowed us to group 89 per cent of the genes we found into functional modules. Our analysis revealed several groups of genes that respond to different environmental conditions, suggesting that *Zygnema* has developed complex regulatory mechanisms to adapt to its surroundings. Our study provides valuable insights into the genome structure, evolution and adaptation of a multicellular streptophyte alga representative of the lineage most closely related to land plants.

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PROJECT NAME

Terrestrialization: Stress Signalling Dynamics in the Algal Progenitors of Land Plants (TerreStriAL)

PROJECT SUMMARY

The multidisciplinary ERC Starting Grant project "Terrestrialization: Stress Signalling Dynamics in the Algal Progenitors of Land Plants (TerreStriAL)" combines evolutionary genomics and molecular biological techniques for the study of the closest algal relatives of land plants to shed light on the evolution of molecular circuits for plant stress response.

PROJECT PARTNERS

Project TerreStriAL is based at the Faculty of Biology and Psychology, Institute for Microbiology and Genetics, Department of Applied Bioinformatics, at the University of Goettingen. Key collaboration partners include several groups from the MadLand consortium (SPP2237) in Germany and groups in the UK, Israel, France, US, Canada, China and beyond.

PROJECT LEAD PROFILE

Born in Germany, Prof. de Vries received his doctorate in Molecular Evolution from the University of Duesseldorf in 2016. After a postdoc in Halifax, Canada, he received an ERC Starting Grant in 2019 to study plant terrestrialisation in light of stress physiology. He is tenured professor since January 2022. Working on questions in plant evolutionary biology, Prof. de Vries has authored over 55 peer-reviewed articles.

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