



Tansley insight

Why we need more non-seed plant models

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Summary

Out of a hundred sequenced and published land plant genomes, four are not of flowering plants. This severely skewed taxonomic sampling hinders our comprehension of land plant evolution at large. Moreover, most genetically accessible model species are flowering plants as well. If we are to gain a deeper understanding of how plants evolved and still evolve, and which of their developmental patterns are ancestral or derived, we need to study a more diverse set of plants. Here, I thus argue that we need to sequence genomes of so far neglected lineages, and that we need to develop more non-seed plant model species.

I. Introduction

Research on animals has for a long time relied on a number of traditional model organisms, such as mouse, fruit fly, zebrafish or worm – all bilaterian Metazoa. One of the reasons for this palette of organisms was the need to have models for human diseases via orthologue studies. In addition to these species, the genomes of which were sequenced early on, the last decade has seen the sequencing of many animal genomes due to their informative phylogenetic position, enabling evolutionary developmental (evo-devo) studies and inference of ancestral states. For example, comparative genomics revealed that the interaction of homeodomain (HD) transcription factors (TF) of the HOX and TALE subfamilies necessary for patterning probably evolved in the last common ancestor of the Eumetazoa (Hudry *et al.*, 2014). Although many sister lineages of bilaterian animals were sequenced and

revealed much, the exact branching order and evolution of the nonbilaterian lineages is still disputed (Lanna, 2015).

The first (small) plant genome to be sequenced was of THE model plant, the weed *Arabidopsis thaliana* (c. 130 Mbp), followed by the c. 500 Mbp (average sized) rice genome. Number three was a tree, poplar, and number four the moss *Physcomitrella patens*. Together with the genome of the unicellular green alga *Chlamydomonas reinhardtii*, comparative genomics allowed us to infer when and how many of the major molecular adaptations of plant evolution had taken place (Rensing *et al.*, 2008). Since then, many plant genomes have been sequenced; however, most of them are angiosperms. Although those cover the majority of extant plant taxa, there are other species-rich lineages as well, in particular ferns, mosses and liverworts. Similar to animal research, it is important to cover the less species-rich, often neglected lineages as well.

II. Evo-devo: inference of how plants evolved

An important change that occurred early in land plant evolution was the establishment of a multicellular diploid 'generation', the sporophyte, and hence the plant-specific alternation of generations. Probably, the sporophyte evolved from dormant zygotes of charophytes by intercalation of mitoses (Lee *et al.*, 2008), leading to the plant version of embryogenesis (see Rensing, 2016, for review). Control genes of haploid (gametophytic) and diploid (sporophytic) generations have been determined by evo-devo studies employing the model moss *P. patens* (e.g. Sakakibara *et al.*, 2013, 2014), in which both multicellular generations are experimentally tractable. More recently, genes involved in the alternation of generations that act at the level of the egg cell or zygote have been identified in the liverwort *Marchantia polymorpha* (Rovekamp *et al.*, 2016) and in *P. patens* (Horst *et al.*, 2016). More and more evidence is emerging that the control of similar structures occurs by orthologues, regardless of the generation in which they are expressed. For example, orthologous transcription factors control the cellular protrusion of structures such as rhizoids and root hairs (Proust *et al.*, 2015), and conducting cells are regulated by the same control genes in mosses and flowering plants (Xu *et al.*, 2014). These examples underline the importance of having data from a diverse set of model organisms that aid evo-devo studies and inference of ancestral traits. Having the genome of these organisms available greatly aids such studies, and the sequencing of these genomes is now feasible due to much reduced sequencing costs.

III. We need more diversity

Currently, Phytozome 12 (<https://phytozome.jgi.doe.gov>) lists 64 genomes of plants and algae, and more are available elsewhere (yet many are version 1 draft genomes, the completeness of which is hard to estimate). The problem is that > 95% of the available plant

genomes are of flowering plants (Fig. 1). Thus, our knowledge of how land plants evolved is severely biased, akin to looking through a distorting lens. Fortunately, the past few years have seen attempts to close the huge gaps in land plant phylogeny by genome sequencing, but projects have not yet been started for all lineages (Fig. 1). *Arabidopsis* is on the extreme fringes of the plant morphospace (Diaz *et al.*, 2016), and *Physcomitrella* is not typical for the majority of mosses. Evo-devo and comparative genomics approaches using only a small set of organisms carry with them a high risk of misinterpretation, because individual species are used as representative for whole clades. In the following I pick three examples to outline how we can profit from more sequenced model organisms.

Conifers

Together with angiosperms, gymnosperm trees (in particular conifers) are an important hallmark of past and current terrestrial habitats. Conifers are interesting in terms of socio-economy because the oldest and largest individuals known are among them. Moreover, they are an evolutionary enigma because a small number of species covers about half of the land masses. Two thirds of the *c.* 1000 gymnosperm species are conifers (Christenhusz *et al.*, 2011). Although the average angiosperm genome is 588 Mbp large, the average gymnosperm genome size is 15.48 Gbp (Kirst *et al.*, 2003), although most of them are not known to be polyploid – the inflated genome size is thus probably mainly due to high transposon activity. Maybe the longevity of the conifers is rooted in different genome structure and function. To date, we do not know much about somatic mutations and epigenetic acclimation of conifers (Avramidou *et al.*, 2015). Yet, if we consider a long-living tree as an assemblage of genetically divergent branches or sections (Burian *et al.*, 2016), it appears feasible that mechanisms of acclimation and of generation of divergent seed banks exist even in individual trees.

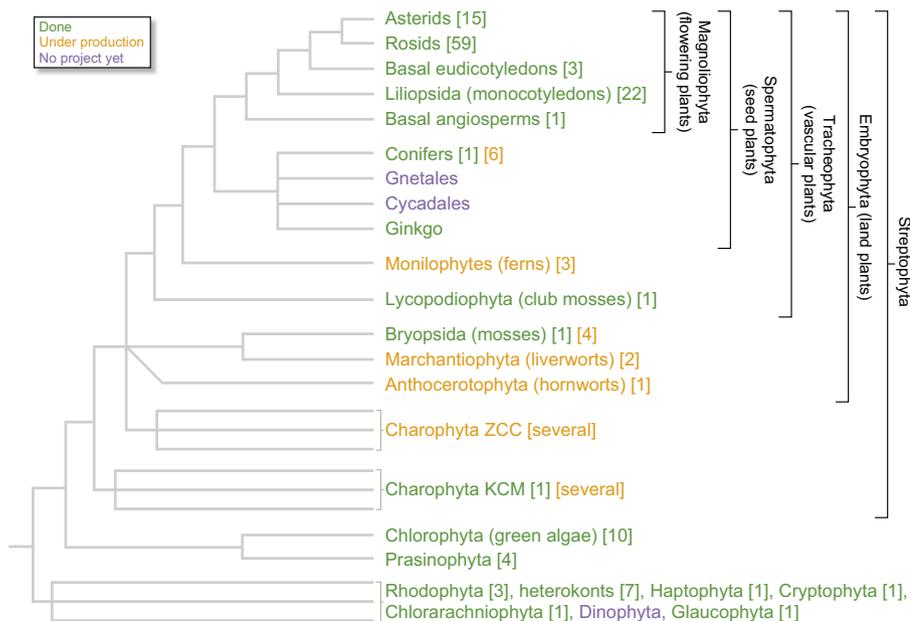


Fig. 1 The plant tree of life. Schematic representation of Streptophyta, rooted on the branch leading to other eukaryotic groups harbouring plastids. The colour code shows species for which the genome has been sequenced and published (green), is under way (orange), or for which there is no project yet (purple). Clades or grades for which the branching order is unclear are shown as multifurcating. Numbers in square brackets show for how many genomes sequencing is currently underway, if any. Brackets to the right mark taxonomic groups.

Due to the large genome sizes, that are also found, for example, in ferns, the first genome (of Norway spruce) was only recently sequenced and published (Nystedt *et al.*, 2013), but several more are works in progress (<http://pinegenome.org/>). Yet, there is more to the gymnosperms than conifers – and the age old question of which of them is sister to the flowering plants is still unresolved (Wickett *et al.*, 2014). Genomic sequences of thus-far neglected lineages (Fig. 1), for example Gnetales and Cycadales, hopefully might help to resolve these issues.

Ferns

Ferns are the second most species-rich lineage of plants and are host to many interesting features such as heavy metal tolerance or Cyanobacteria symbiosis for nitrogen fixation. Moreover, ferns are sister to seed plants and thus, for understanding seed plant-specific evolutionary innovations, ferns are the natural outgroup. As an example, it has recently been shown that abscisic acid (ABA) controls sexual reproduction in the fern *Ceratopteris richardii*, and that the conserved ABA signaling pathway was only later co-opted to control seed dormancy and stomatal movement (McAdam *et al.*, 2016). Ferns have recently been discussed as models for evo-devo approaches to understand the evolution of shoot development (Plackett *et al.*, 2015), and have been used to determine commonalities of leaf evolution and development of vascular plants (Vasco *et al.*, 2016). Several ferns are now being sequenced and initial low-pass sequencing has already provided glimpses at peculiar genome structures (Wolf *et al.*, 2015). In particular, *Azolla* and *Salvinia* are being sequenced and ‘... differ dramatically in genome size, life history, and habit, and thus represent the immense diversity of extant ferns’ (Sessa *et al.*, 2014). The availability of fern genomes will certainly much improve our understanding of how important features of vascular plants (e.g. vasculature in the narrow sense), and of seed plants (e.g. seeds and flowers) evolved.

Charophytes

Several recent papers focus on the water-to-land transition of plant life. Intriguingly, some charophyte algae (cf. Box 1) share molecular features with land plants that were crucial for the conquest of land. Although such features were previously thought to have been acquired concomitant with the transition, it becomes more and more apparent that many evolved in the freshwater charophyte algae. For example, a peculiar set of plastid genes were transferred to the nucleus, and polyplastidy established, in the ZCC grade (cf. Box 1) of streptophyte algae (de Vries *et al.*, 2016). Moreover, the peculiar mode of cell division that enables land plant 3D growth is rooted in streptophyte algae. The phragmoplast probably evolved in the last common ancestor of the ZCC grade, whereas the preprophase band apparently evolved in the Zygnematales (Buschmann & Zachgo, 2016), the closest sister lineage to land plants (Wickett *et al.*, 2014). With regard to the plant-type cell wall, rosette-forming cellulose synthase complexes are already present in the KCM grade, and members of this grade, such as Klebsormidium, show species that can cope with terrestrial habitats (Hori *et al.*, 2014). Potentially, charophytes might have made the

step to land, including an altered cell wall, and land plants evolved after this transition (Harholt *et al.*, 2016). Although Zygnematales are the closest relatives of land plants, they were apparently secondarily reduced through the course of evolution (Delwiche & Cooper, 2015), leading, for example, to their peculiar form of sexual reproduction – conjugation. Charales, however, are morphologically most complex and similar to land plants, but some of their features, such as rhizoids controlled by strigolactones (Delaux *et al.*, 2012), might represent evolutionary inventions that occurred after the divergence from the last common ancestor with other ZCC lineages.

IV. Genomes are necessary, but not sufficient

Genome sequences are very useful for comparative genomics and phylogenomics, and the basis for evo-devo approaches. However, it is also important that the organisms in question are genetically tractable, easy to culture *in vitro*, and that the whole life cycle – including sexual reproduction – can be followed under standardized conditions. For seed plant species, all these required features are not typically an issue (although, e.g., the high generation time of tree species makes typical genetic approaches cumbersome). Recently, transformation protocols for ferns have been published (Muthukumar *et al.*, 2013) as well as for the charophyte alga *Penium* (Sorensen *et al.*, 2014), although their efficiency might be sub-optimal. Approaches to transform other charophytes using, for example, particle gun or microinjection, which has been

Box 1 Glossary

Bryophytes

Encompass the three lineages mosses, hornworts and liverworts. Bryophytes' relationship to vascular plants is unresolved, as is the question of whether they are monophyletic. The most recent phylogenomic analyses describe the mosses and liverworts as monophyletic and the hornworts potentially as sister to all other land plants.

Charophyta

Or streptophyte algae, a grade consisting of six extant lineages (see KCM and ZCC grade). Some Charophyta share a common ancestor with the land plants.

KCM grade

Three lineages of Charophyta, encompassing Klebsormidiales, Chlorokybales and Mesostigmatales, that are sister to the ZCC grade and the land plants.

Streptophyta

Unite the Charophyta with the land plants (Embryophyta).

ZCC grade

Three lineages of Charophyta, encompassing Zygnematales, Coleochaetales and Charales, that are sister to land plants. The Zygnematales are most probably the closest sister lineage to land plants.

traditionally applied to *Chara* (Oikawa *et al.*, 2011), are being developed. CRISPR/Cas9-derived technology will certainly make genomic modification attempts easier than they used to be. By making these organisms genetically tractable, evo-devo approaches utilizing loss-of-function mutants (such as knockout of leaf and shoot control genes in ferns) and complementation assays (e.g. using land plant rhizoid control genes in charophytes) will become feasible. Axenic *in vitro* culture of ferns (Hanke & Rensing, 2010) as well as charophytes (Kato *et al.*, 2008), including completion of the life cycle, is possible (Fig. 2). For bryophytes (cf. Box 1), axenic *in vitro* culture and completion of the life cycle is not an issue (Beike *et al.*, 2010). Although many approaches have thus already been undertaken to develop flagellated (non-seed) plant model organisms, much more work is needed. For many of the organisms that are currently being sequenced, the palette of molecular tools and

in vitro cultivation practices is not yet established. Axenic cultivation is especially important to study the influence of microbiota on growth and development under standardized conditions. For example, axenic Charales cultures would allow the study of the influence of fungi and bacteria on formation of rhizoids or on germination of dormant spores.

V. What do we need?

The still existing gaps in the land plant phylogeny need to be closed (Fig. 1). Starting from the earliest divergences within the streptophytes, we need representatives for all six charophyte lineages, KCM and ZCC, as outlined earlier. From KCM there is the *Klebsormidium flaccidum* genome (Hori *et al.*, 2014), from ZCC there are projects for Zygnematales as well as *Chara braunii* (Fig. 2).

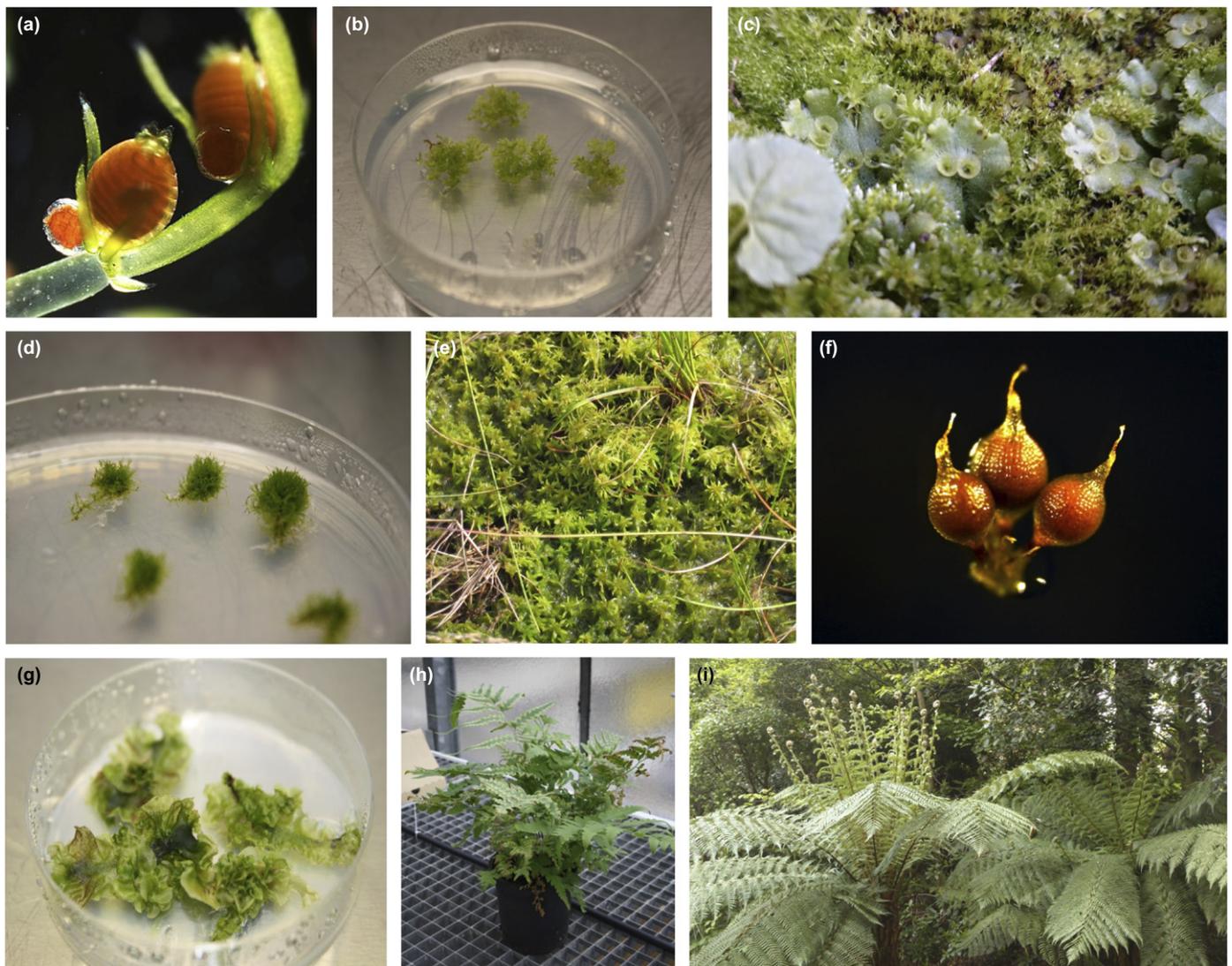


Fig. 2 Flagellated plants. Images of flagellated plants (having motile gametes), also known as non-seed plants or spore plants. For most of them the haploid gametophytic generation is dominant. In ferns, both generations are free-living. In charophytes, the diploid phase is represented exclusively by the zygote. (a) *Chara braunii* gametangia, note the oospore with its characteristic structure; (b) *Anthoceros formosae*, gametophyte; (c) *Marchantia* thallus (gametophyte) with gemmae cups; (d) *Takakia lepidozoides* gametophyte; (e) *Sphagnum* (peat moss) gametophyte; (f) *Physcomitrella patens* sporophytes; (g) *Microlepidia cf. marginata* (fern) gametophyte and (h) sporophyte; (i) tree fern *Dicksonia*. Images courtesy of Marco Göttig (b, d, g, h), Rabea Meyberg (f), Melanie Barth (a) and Stefan Rensing (c, e, i).

In particular, the comparative analysis of ZCC species will allow us to infer which characters evolved in their last common ancestor (such as branching, polyplastidy and the phragmoplast), vs which were secondarily reduced in Zygnematales (such as flagellae), arose independently in Charales (like rhizoids), or are common only to land plants and their closest sister lineage (like the preprophase band).

Although most transcription factor families evolve by paralogue retention, it has been shown that this is not always the case (Sayou *et al.*, 2014). The inferences made in this study would have been rendered easier if more bryophyte genomes would have been available, and their branching order resolved. *Anthoceros agrestis* will probably be the first hornwort genome to be published (Szoenyi *et al.*, 2015); more are needed. The analysis of hornwort genomes will hopefully allow us to determine definitively which of the bryophyte lineages is sister to all land plants, a crucial piece of information that affects all inferences of evo-devo studies.

The *M. polymorpha* genome is sequenced but as yet unpublished. More genomes are needed to cover the diversity of liverworts, for example, from the Haplomitriopsida and Jungermanniopsida. An important analysis to be carried out is the question of how Marchantiidae are able to cope with the secondary loss of organellar RNA editing (Groth-Malonek *et al.*, 2007) that is a hallmark of land plants.

Within the mosses, two of the three earliest splits are now being covered by sequencing *Sphagnum* and *Takakia lepidozoides*—there is no *Andreaea* project yet. The branching order of these basal lineages of mosses is unresolved, the analysis of the genome sequences will hopefully allow us to infer which of them is sister to all other mosses.

With *Ceratodon purpureus* a moss with sex chromosomes is being sequenced, and with *Funaria hygrometrica* a relative of *P. patens* that features a much more elaborate sporophyte. They will allow the study of evolution and mechanistics of sex chromosomes in haploid organisms, respectively of sporophytic features as a function of niche adaptation.

Within the lycophytes the spike moss *Selaginella moellendorffii* has been sequenced (Banks *et al.*, 2011), but no representative of Isoetales (quillworts) and Lycopodiaceae (club mosses). From the moniliformopses, the polypod ferns *Salvinia*, *Ceratopteris* and *Azolla* are being sequenced, but there are also for example Equisetales (horsetails), Ophioglossaceae (adder's tongues) or Psilotaceae (whisk ferns). As mentioned earlier, many lineages of nonconifer gymnosperms are yet to be covered, for example cycads or Gnetales. In all of these cases, the genome sequences of neglected lineages offer the potential to determine thus far unresolved branching orders, and to infer how their morphological and developmental characteristics evolved.

VI. Conclusions

The past decade has seen an exciting explosion of plant genome sequences, which have sparked interest in so far atypical models for evo-devo research. Some ancestral features of land plants already have been inferred in the past few years based on such data and models, but much more needs to be learned. Similar to

animals (Goldstein & King, 2016), it is important to develop models based on their phylogenetic position and morphological or cellular peculiarities, rather than their (initial) experimental tractability. By developing more models, and by sequencing and comparing more genomes of so far neglected lineages, we will gain a much better understanding of land plant evolution. This is doubtless necessary, in particular to understand how plant life will respond evolutionarily to changing environmental conditions.

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