The Evolution of Complex Multicellularity in Streptophytes

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Abstract

The streptophyte lineage of green plants, comprised of charophyte algae and land plants, inhabits freshwater ecosystems and dominates nearly every terrestrial environment. Land plants form a clade within a paraphyletic assemblage of charophyte algae comprising a phylogenetic grade implying land plants evolved from an ancestral freshwater alga. Within the streptophyte lineage of green plants, the evolution of complex multicellular bodies in both haploid and diploid generations is unique to the lineage of land plants. The grade of charophyte algal lineages, on the other hand, exhibit a gradient of increasing complexity from unicellular, simple multicellular to relatively complex multicellular gametophyte, the haploid gamete producing generation, but always retain a unicellular sporophyte, the diploid spore producing generation. Here we discuss the origins of multicellularity in streptophytes, and examine streptophyte characters that pre-date the origin of land plants, and which may have helped facilitate the evolution of a complex multicellular form in both generations during the colonisation of land. We conclude by examining molecular genetic innovations underpinning complex tissue formation in land plants and considering whether the complex multicellularity in sporophyte generation of land plants evolved *de novo* or by co-option of an ancestral gametophyte program.

16.1 The early diverging streptophytes – unicellular and simple multicellular algae

Embryophytes (land plants) are nested within a grade of charophyte algae, and together these taxa comprise the Streptophytes (Figure 16.1). Charophyte algae inhabit freshwater environments, and it is their gametophyte generations, which range from unicellular to complex multicellular, that are predominantly observed. Their diploid generation is always unicellular, and is often a resting, or dispersal, stage of the life cycle. A lineage uniting *Mesostigma* and *Chlorokybus* is positioned as sister to all other Streptophytes (Lemieux *et al.* 2007; Puttick *et al.* 2018; Timme *et al.* 2012; Wang *et al.* 2020; Wickett *et al.* 2014). *Mesostigma* is an aquatic freshwater alga with a unicellular body plan, and *Chlorokybus* occupies alpine terrestrial habitats and consists of loose packets of cells (Geitler 1942; Lauterborn 1894; Marin and Melkonian 1999). Many features of *Mesostigma*, namely its unicellular, motile vegetative phase and freshwater habitat are characters likely shared with the ancestral streptophyte (Leliaert *et al.* 2012; Umen 2014).

Along with land plants, extant members of all four other lineages of charophyte algae possess multicellular gametophytes, with flagella lost in the vegetative stages but maintained — with the exception of Zygnematophyceae taxa — in the zoospores (i.e. motile unicellular spores) (Stewart and Mattox 1975). Klebsormidiophyceae are usually placed as sister to a clade including a grade of three groups of charophyte algae (Charophyceae, Coleochaetophyceae and Zygnematatophyceae) and Embryophytes. Collectively this group has been named the Streptophytina (Lewis and McCourt 2004). Thus, simple multicellularity likely evolved in the common ancestor of the Klebsormidiophyceae and the Streptophytina (Fig. 16.1).

Extant Klebsormidiophyceae taxa are characterised by unbranched filamentous, such as *Entransia* and *Klebsormidium* species, or sarcinoidal forms (i.e. three-dimensional packets of cells) as in some species of *Interfilum*, growing in shallow freshwater or on damp surfaces (Mikhailyuk *et al.* 2008, 2018; Rindi *et al.* 2008). Given that *Entransia* and *Hormidiella*, two unbranched filamentous genera, diverge from basal nodes within the Klebsormidiophyceae (Mikhailyuk *et al.* 2008, 2018), it is probable that the ancestor had an unbranched filamentous morphology, with sarcinoidal forms derived. Extant Klebsormidiophycean algae

have a simple rather than complex multicellular form (following Knoll (2011)), with diffuse cell divisions, and with filaments generally lacking differentiated or specialised cells, apart from differentiation between reproductive and vegetative cell types (Cook and Graham 2017; Sluiman *et al.* 1989). Additionally, Klebsormidophyceae taxa lack plasmodesmata (Lokhorst 1996; Mikhailyuk *et al.* 2014; Stewart *et al.* 1973) — cytoplasmic connections between cells — suggesting that intercellular communication is less critical for development in this lineage. Given the taxonomic position of Klebsormidiophyceae, the earliest multicellular streptophytes — dating to around 800-600Ma (Morris *et al.* 2018) — were possibly comprised of simple unbranched filaments in the gametophyte generation, with this growth form retained by most extant Klebsormidiophyceae taxa (Leliaert *et al.* 2012; Umen 2014). The unbranched filamentous form may have been selectively advantageous, allowing for more efficient nutrient foraging via growth along vectors and additionally reducing the adverse effects of grazing predation by conferring greater size (Niklas 2000; Umen 2014).

Embryophytes

apical cell with 3+- cutting faces
multicellular sporophyte

515-470Ma

Vascular plants

Mosses

Liverworts

Hornworts

pre-prophase bands

charophyte algae

reduced complexity
lost plasmodesmata
lost anisogamy
lost matrotrophy

Zygnematophyceae

matrotrophy?

Coleochaetophyceae

apical cell?
anisogamy?
plasmodesmata?
phragmoplast

multicellular gametophyte
non-motile vegetative stage

Charophyceae

Klebsormidiophyceae

890-673Ma

Mesostigma

Chlorokybus

Chlorophytes

Lycopodium (2n)

(n)

(2n)

(2n)

Isophysis

(n)

Andreaea

Sphagnum

(n)

(2n)

(2n)

(n)

Marchantia

Haplomitrium

(2n)

Anthoceros (n)

Spirogyra (n)

Spirogloea (n)

Coleochaete (n)

Chaetosphaeridium (n)

Chara (n)

Klebsormidium (n)

Mesostigma (n)

(2n)

(n)

Chlamydomonas
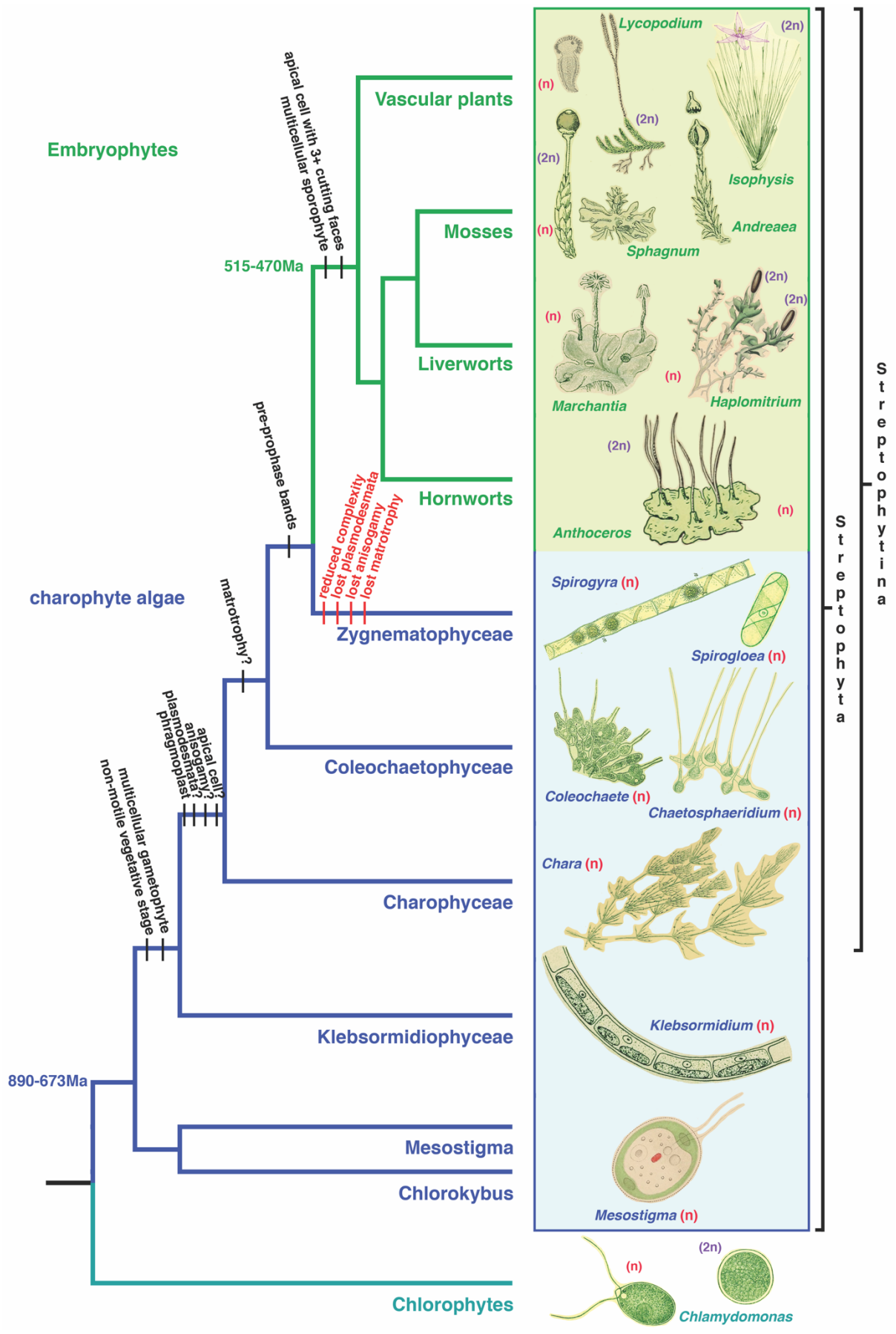
Streptophytina

Streptophytina

Streptophyta

4

Figure 16.1: Phylogenetic relationships of the streptophytes. Streptophytes (green and dark blue branches) form a monophyletic group diverging as sister to Chlorophytes (light blue branch). Within Streptophytes, the Embryophytes (green branches) resolve as a monophyletic group nested within a phylogenetic grade of charophytes (dark blue branches). Putative character acquisitions (in black) and losses (in red) indicated at nodes are inferred by parsimony. The ploidy of life cycles stages for representative taxa are indicated in brackets (n, red; 2n, purple), with the unicellular diploid sporophytes of charophyte taxa not shown. Phylogenetic relationships follow Puttick *et al.* (2018) and node age estimates are based on Morris *et al.* (2018). Images: (Allen 1888; De Bary 1858; Collins 1909; Coupin 1911; Gottsche 1843; Hooker 1837; Klebahn 1914; Klebs 1896; Lauterborn 1899; Lendner 1911; Strasberger et al. 1911).

16.2 The Streptophytina and anisogamy – a clue into the origins of multicellularity

Recent phylogenetic analyses with broader taxon coverage place Zygnematophyceae as sister to Embryophytes, with Coleochaetophyceae sister to Zygnematophyceae + Embryophytes, and Charophyceae more distantly related (Fig. 16.1) (Finet *et al.* 2010; Wickett *et al.* 2014). Extant Charophyceae, such as the stoneworts *Nitella* and *Chara*, have large gametophyte bodies composed of united branched filaments, occupying deep and generally permanent freshwater habitats. The diversity of reproductive morphologies observed in the fossil record suggests that extant Charophyceae only represent a small fraction of previous family diversity (Feist *et al.* 2005). Extant Coleochaetophyceae are comprised of branched filamentous and discoidal forms (Delwiche *et al.* 2002; Hall and Delwiche 2007; Thompson 1969), with the ancestral form predicted to be a branched filament. Within Zygnematophyceae there are both unicellular and filamentous taxa, with filamentous forms represented by the basally diverging genera *Spirogyra*, *Mougeotia* and *Mesotaenium*, and desmids, unicellular algae composed of two rigid halves that share a nucleus, derived (Hall *et al.* 2008). However, the recent placement of the unicellular genus *Spirogloea* as sister to all other members raises the possibility that the ancestral Zygnematophyceae was unicellular (Cheng *et al.* 2019).

Along with land plants, multicellular gametophytes are present in extant members of in the four most recently diverging lineages of the charophyte algae grade (Fig. 16.1), suggesting that multicellularity may have evolved just once in the ancestor of Klebsormidiophyceae and Streptophytina. However, the potential for a unicellular ancestral state of the Zygnematophyceae allows for the possibility of a unicellular algal ancestor of land plants (Stebbins and Hill 1980).

Of the Streptophytina, Embryophytes, Charophyceae and Coleochaetophyceae are anisogamous and oogamous, suggesting that these traits may be plesiomorphic (i.e. ancestral), with both characters subsequently lost in the Zygnematophyceae, which sexually reproduce via conjugation of cells between or within filaments (reviewed in Mori *et al.* 2015). The transition from uni- or simple multicellularity to complex multicellularity in eukaryotic lineages has been proposed to drive disruptive selection resulting in dimorphic gametes (i.e. anisogamy) (Parker *et al.* 1972; Bulmer and Parker 2002; Hanschen *et al.* 2018). Most (but not all) unicellular organisms are isogamous whereas multicellular organisms can more readily evolve anisogamy (Bulmer and Parker 2002; Hanschen *et al.* 2018). If multicellularity had evolved multiple times independently during the evolution of Streptophytina lineages, then it might be expected that anisogamy would be a homoplasious (independently acquired) trait regulated by distinct genetic factors. However, the DUO POLLEN1 (DUO1) transcription factors, which regulate sperm differentiation in

Embryophytes, are thought to have a homologous role in sperm differentiation of the Charophyceae, since *Chara braunii DUO1* orthologs can rescue Mp*duo1* knockouts in the liverwort *Marchantia polymorpha* (Higo *et al.* 2018). *DUO1* orthologs have been lost from Zygnematophyceae, which is consistent with these charophyte algae having lost anisogamy, instead sexually reproducing via conjugation of distinct mating-type cells (Higo *et al.* 2018; Hisanaga *et al.* 2019). Thus, given that anisogamy is likely a homologous trait among the Streptophytina, multicellularity probably evolved only once in the common ancestor of the Streptophytina, and by parsimony in the common ancestor of the Streptophytina + Klebsormidiophyceae. As discussed below, multicellularity in the Streptophytina - with the exception of the Zygnematophyceae – is complex, with differentiation of specialized vegetative cell types in the gametophyte (as well as the sporophyte in Embryophyta). Thus, complex multicellularity likely evolved in the gametophyte generation in the ancestral Streptophytina.

16.3 The Streptophytina – increasing morphological complexity: phragmoplasts, branching, apical cells, specialised tissues and plasmodesmata.

In *Mesostigma, Chlorokybus* and Klebsormidiophyceae, cytokinesis occurs via centripetal cleavage without the formation of a cell plate (Lokhorst *et al.* 1988; Manton and Ettl 1965; Pickett-Heaps 1975; reviewed in Buschmann and Zachgo 2016). In contrast, the Streptophytina possess efficient microtubule arrays called phragmoplasts, which assemble the cell plate and more effectively allow for shifts in the plane of cell division (Pickett-Heaps 1975; Pickett-Heaps *et al.* 1999). In Charophyceae, Coleochaetophyceae and some Zygnematophyceae (i.e. *Mougeotia*), the action of phragmoplasts facilitates the formation of branched filaments by rotations in the planes of division of filament cells (reviewed in Buschmann 2020). Additionally, preprophase bands of microtubules, which effectively mark the site of the next plane of cell division, originated in the ancestor of Zygnematophyceae and Embryophyta (Buschmann and Zachgo 2016). The acquisition of pre-prophase bands in the ancestor of Zygnematophyceae and Embryophyta would again suggest that their common ancestor was not unicellular, but rather multicellular, with cell divisions occurring in multiple dimensions.

Unlike the Klebsormidiophyceae, where growth is diffuse, Streptophytina have evolved localised growth from apical cells (Graham *et al.* 2000; Leliaert *et al.* 2012). However, as discussed later, it remains uncertain as to whether or not the apical cells of charophytes and Embryophytes are homologous. The ongoing development of a range of charophyte and Embryophyte model organisms (Domozych *et al.* 2016) provides the opportunity for determining whether a conserved genetic program regulates the apical cells of charophytes and Embryophytes. The apical cells of the Charophyceae, Zygnematophyceae and some Coleochaetophyceae divide anticlinally, and do not directly undergo rotations in plane division; instead, the subapical cells are the sites of lateral bulging and/or periclinal divisions giving rise to uniseriate branched filaments (Buschmann 2020; Delwiche *et al.* 2002; Graham *et al.* 2000). An exception is the majority of *Coleochaete* species, where the apical cell itself cuts from two faces to give rise to apically branching filaments or discoidal body plans (Delwiche *et al.* 2002; Graham *et al.* 2000). However, this character may be derived within Coleochaetophyceae rather than ancestral, given that early diverging lineages tend to display subapical branching (i.e. *Chaetosphaeridium* spp. and *Coleochaete irregularis*) (Delwiche *et al.* 2002; Thompson 1969). Embryophyte apical cells are unique among the Streptophytina in cutting from three or more faces and thus directly co-ordinating

the establishment of three-dimensional tissues and body plan (Bowman *et al.* 2019; Campbell 1918; reviewed in Moody 2020).

Coinciding with the emergence of bodies controlled by apical cells in the Streptophytina is an increase in the number of specialised cell and tissue types (Figure 16.2). For example, the early diverging Charophyceae genera are branched filaments attached to the substrate by multicellular rhizoids, with filaments consisting of central axes of multinucleate internodal cells separating whorls of branchlets, which radiate from clusters of uninucleate nodal cells (Beilby and Casanova 2014; Wood and Imahori 1965). Additionally, members of the Coleochaetophyceae possess specialised seta cells, along with maternal cells that envelope the zygote following fertilization (Fig. 16.2) (Marchant 1977; Pringsheim 1860). Likewise, some Zygnematophyceae taxa (*Mougeotia* and *Spirogyra* spp.) have rhizoids and/or rhizoid-like tip cells (Buschmann 2020; Inoue *et al.* 2002; Nagata 1973; Pascher 1906).
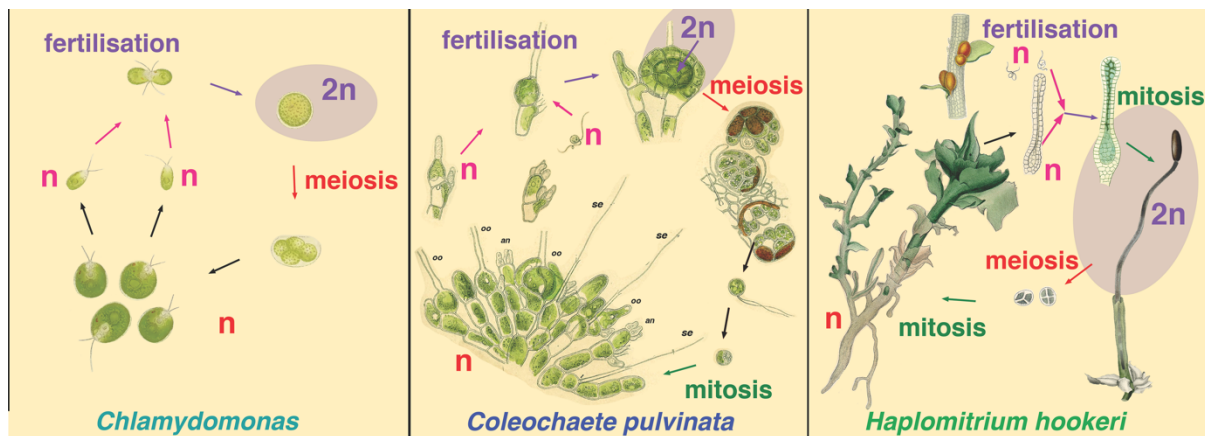

Figure 16.2: Increased morphological complexity in the life cycles of the streptophytes. Depicted are the life cycles of the unicellular chlorophyte *Chlamydomonas* sp., the charophyte *Coleochaete pulvinata* and the liverwort *Haplomitrium hookeri*. Vegetative gametophyte stages = n (red), gametes = n (pink) and sporophyte stages = 2n (purple). In *Chlamydomonas,* both stages of the life cycle are unicellular, with gametes fusing to produce a zygote that immediately undergoes meiosis to form spores that develop into the vegetative cells of the next generation. By parsimony, the ancestral streptophyte life cycle likely resembled the life cycle of *Chlamydomonas*. Members of the Streptophytina (Charophyceae, Coleochaetophyceae and Embryophyta) have evolved increased morphological complexity, with multiple specialised cell types and growth from apical cells. The charophyte alga *C. pulvinata* possesses a multicellular gametophyte, with growth controlled by localised apical cells, and produces specialised cell types such as seta (*se*) and gametangia (antheridia, *an* and oogonia, *oo*), with the latter producing dimorphic gametes. After fertilisation, the enlarged zygotes are protected by specialised enveloping cells, with the zygote undergoing cell divisions to produce 8-32 meiospores per fertilisation event (Graham 1984). These meiospores disperse, and after settling on a substrate undergo mitoses (green) to generate a multicellular haploid plant. The liverwort *H. hookeri,* like all extant land plants, displays complex multicellularity in both generations, undergoing mitoses following both meiosis and fertilization. The gametophyte develops into a three-dimensional axial shoot, with gametangia producing sperm and eggs. After fertilisation, an unbranched, axial sporophyte develops which is attached and nutritionally dependent on the gametophyte generation. Images: (Goroschankin 1891; Gottsche 1843; Pringsheim 1860)

The evolution of localised apical cells and cell specialisation suggests the requirement of increased cell-to-cell communication during development. All Streptophytina lineages except the Zygnematophyceae contain taxa with plasmodesmata — i.e. membrane-lined channels through cell walls (Cook *et al.* 1997; Marchant and Pickett-Heaps 1973; Pickett-Heaps 1967a, 1967b). Some authors suggest that charophyte algae and Embryophyte plasmodesmata are homologous (Graham *et al.* 2000; Nicolas *et al.* 2018; Raven 2005), while others propose that they evolved in parallel (Brunkard and Zambryski 2017). Plasmodesmata in bryophytes and vascular plants have central strands of compressed endoplasmic reticulum (i.e. desmotubules), a character not observed in studies of *Coleochaete* species (Cook and Graham 1999; Cook *et al.* 1997; Stewart *et al.* 1973). Some studies have indicated that plasmodesmata in *Chara* cells occasionally have desmotubules or desmotubule-like central structures (Brecknock *et al.* 2011; Cook *et al.* 1997), while others have proposed that they are absent (Franceschi *et al.* 1994). The presence of plasmodesmata in the ancestral Streptophytina (or otherwise evolving in parallel shortly afterwards) would allow for metabolite and resource sharing, and additionally facilitate efficient transport of signalling molecules along spatial gradients, promoting cell specialisation (Hernández-Hernández *et al.* 2012; Lucas and Lee 2004; Niklas and Newman 2013).

16.4 The advent of a terrestrial flora

There exists evidence of a widespread land flora of Embryophyte affinity by the mid-Ordovician (470 MYA) in the form of meiotic cryptospores — fossilised spores with walls containing sporopollenin, but distinct from monolete and trilete spores and pollen grains — that in some respects resemble those produced by extant bryophytes (Edwards *et al.* 2014; Wellman and Gray 2000). Despite the abundant cryptospore assemblages, there is little fossil evidence of the plants that produced the cryptospores; however, it is likely they were produced inside complex multicellular sporophytes although it is possible that Embryophytic spores preceded the evolution of multicellular sporophytes (Brown and Lemmon 2011; Strother and Taylor 2018). Regardless, the common ancestor of all extant land plants possessed complex multicellular bodies in both haploid gametophyte and diploid sporophyte generations. Thus, in the 100 million or so years after their divergence from the most closely related lineage of extant charophycean algae, the lineage leading to land plants experienced two key innovations with respect to body plan. First, the evolution of a meristem containing an apical cell with three or more cutting faces enabled the development of complex multicellularity with three-dimensional tissues. A three-dimensional body plan with a differentiated epidermis surrounding parenchymatous tissues facilitated adaptation to the terrestrial environment where water is limiting. Second, complex multicellularity with three-dimensional tissues evolved in both gametophyte and sporophyte generations. It has been proposed that the adaptive significance of the evolution of a multicellular sporophyte is the potential generation of thousands of haploid spores from a single fertilization event, presumed to be limiting in the terrestrial environment, rather than just four haploid progeny per fertilization event in the ancestral alga (Bower 1908). These two key evolutionary innovations, along with the establishment of arbuscular mycorrhizal interactions with fungi, have been credited for the rapid radiation of early land plants (Field *et al.* 2015; Selosse and Le Tacon 1998).

That land plants evolved complex multicellularity in both gametophyte and sporophyte generations raises two key questions. First, what were the molecular genetic innovations that facilitated the evolution of complex multicellularity? Second, did the

evolution of complex multicellularity evolve in one generation initially, with genetic programs subsequently co-opted by the second generation, or did complex multicellularity evolve *de novo* independently in each generation? While these two questions may be inextricably linked we will discuss them in series below.

16.5 How did complex tissue formation evolve?

As land plants evolved from an ancestral charophyte alga that possessed a life cycle similar to that of extant relatives, with a multicellular gametophyte generation and a single-celled (zygote) sporophyte generation, it has been proposed that the gametophyte generation is the 'older' multicellular generation (Bower 1908). While apical growth and apical cells evolved within the charophyte algal grade, given the sister relationship between the Zygnematophyceae and Embryophytes (Figure 16.1), it is not clear that they are homologous with apical cells in land plant gametophytes. In contrast, parsimony indicates gametophyte shoot apical meristems, and their apical cells, are homologous throughout Embryophytes (Bowman *et al.* 2019). As alluded to above, a key innovation was the evolution of apical cells with three or more cutting faces producing three-dimensional tissues (Graham *et al.* 2000). The apical cells and their immediate derivatives that also actively undergo division in multiple planes act as a pool of stem cells, a meristem, from which all the other tissues and organs of the plant body are ultimately derived. Development from apical meristems allows directional growth whose vector is influenced by light (phototropism) and gravity (gravitropism). Directional growth is another adaptation to terrestrial habitats, where substantial spatial differences in environmental stimuli exist, in contrast to the relative uniformity of aqueous environments.

Following spore germination, the gametophyte generations of bryophytes and ferns undergo a constitutive, or inducible depending upon light conditions, protonemal growth period, where one-dimensional algae-like filaments are formed, before transitioning to two-dimensional growth consisting either of branched protonemata or prothallus body plans (Campbell 1918; Goebel 1905). The duration of two-dimensional growth may be extended (mosses) or brief (most other lineages) following which three-dimensional growth is established. During this time, either in the two-dimensional prothallus or at the onset of three-dimensional growth, an apical cell with three or more cutting faces is specified. The precise anatomies of gametophyte shoot meristem apical cells vary among the different major lineages of land plants, but all involve shifts in division plane between successive cell divisions. The continual shifts in apical cell division planes and subsequent pattern formation imply substantial cell communication networks act between apical cells and other cells within the meristem. One form of communication is via plasmodesmata, which allow small cytoplasmic molecules to move between cells. In both fern and liverwort gametophytes plasmolysis causes severance of plasmodesmatal connections (as well as other disruptions) resulting in cell dedifferentiation and subsequent reestablishment of new apical cells (e.g. Nagai 1919; Tilney *et al.* 1990). Likewise, if an apex including its apical cell is removed by decapitation, a new apical cell is specified nearby, indicating that signals emanating from the apical cell prevent other nearby cells from becoming apical cells (e.g. Vöchting 1885; Nishihama *et al.* 2015).

16.6 Evolution of new signalling pathways facilitated evolution of the gametophyte shoot meristem

A direct approach to uncover the genetic underpinnings of apical cell formation and function is to screen for mutants in which this process is disrupted. Such an approach has been undertaken in the moss *Physcomitrium* (*Physcomitrella*) *patens*, uncovering a number of genes whose activity is required for proper gametophyte apical cell function (Moody *et al.* 2018). As the functions of most the genes identified have not been investigated in other taxa, and since this approach has been recently reviewed (Moody 2020; Véron *et al.* 2020), here we focus on a second approach — an investigation of candidate molecules based on the extensive knowledge of angiosperm sporophyte apical meristems.

Arguably the most important land plant hormone is auxin, with the auxin-mediated transcriptional responses as first characterized in angiosperms being a land plant innovation (Bowman *et al.* 2017; Mutte *et al.* 2018). In the liverwort *Marchantia polymorpha*, loss of the primary auxin biosynthetic pathway, that mediated by the TAA and YUCCA enzymes, results in a complete loss of developmental patterning in the gametophyte, with the mutant plants reduced to globular masses of undifferentiated cells (Eklund *et al.* 2015). Both TAA1 and YUCCA2, the only gametophyte-expressed members of their respective gene families, are expressed in the apical cell and its immediate derivatives (Eklund *et al.* 2015). Likewise, in *P. patens*, gametophyte apical stem cells synthesize auxin, but the apical cell and its immediate derivatives themselves are insensitive to auxin, with auxin signalling present only more distally in cells that enter differentiation pathways (Landberg *et al.* 2021). These observations are consistent with the hypothesis that the evolution of auxin signalling was instrumental in the evolution of focal growth from meristems as opposed to more diffuse growth observed in many charophyte lineages (Flores-Sandoval *et al.* 2018). While local auxin synthesis and subsequent downstream signalling are critical for gametophyte shoot meristem formation and function, PIN-FORMED1 (PIN)-mediated polar transport of auxin has not yet been shown to be required for the formation or maintenance of the gametophyte shoot meristem, despite its importance in sporophyte shoot organization (Viaene *et al.* 2014). This implies that localized auxin synthesis and limited, perhaps diffusion-based, auxin movement along with a spatially localized response is sufficient for gametophyte shoot meristem function. Auxin-mediated signalling was integrated into a pre-existing transcriptional network and acts as a facilitator of other transcriptional networks rather than specifier of cellular fates in land plants (Bennett and Leyser 2014; Flores-Sandoval *et al.* 2015; Stewart and Nemhauser 2010). Hence, it is critical to identify the pre-existing networks with which it was integrated and those with which it co-evolved in order to understand the origin of the meristem.

A second class of signalling pathway crucial for meristem function also evolved in the ancestral land plant, with receptor kinase-peptide ligand signalling pathways greatly expanding in the ancestral land plant (Bowman *et al.* 2017). One class, composed of CLAVATA3/EMBRYO SURROUNDING REGION-related (CLE) peptides and associated CLAVATA1/TDIF RECEPTOR (CLV/TDR) receptors, was originally identified as negative regulators of stem cell activity in angiosperm sporophyte meristems (Somssich *et al.* 2016). Using a reverse genetic approach in *P. patens*, CLE peptide signalling was demonstrated to be critical for proper cell division plane reorientation that occurs during the transition from two- to three-dimensional growth (Whitewoods *et al.* 2018). While it was reported that *P. patens* CLE loss-of-function mutants exhibit over-proliferation phenotypes, intriguingly, application of exogenous CLE peptide to *P. patens* gametophyte shoots stunted their growth (Whitewoods *et al.* 2018). A similar reverse genetic approach undertaken in *M. polymorpha* also revealed a role for CLE signalling in gametophyte shoot meristem function. In *M. polymorpha*, application of exogenous CLE peptide causes an accumulation of stem cells in the shoot meristem, while Mp*cle2* loss-of-function alleles exhibit a reduction in meristem

size (Hirakawa *et al.* 2020). These data clearly demonstrate that MpCLE2 signalling in the *M. polymorpha* meristem acts to stimulate stem cell proliferation (or restrict differentiation), an effect opposite to that observed in angiosperm sporophyte shoot meristems (Hirakawa *et al.* 2020). Furthermore, a key downstream target of CLE signalling in sporophyte meristems, a WUSCHEL-relared homeobox (WOX) transcription factor, is not a downstream target of CLE signalling in bryophyte gametophyte shoot meristems (Hirakawa *et al.* 2020; Sakakibara *et al.* 2014). However, another class of transcription factor critical for sporophyte shoot meristem function, AINTEGUMENTA/PLETHORA/BABY BOOM (APB), does play a critical role in the establishment of *P. patens* three-dimensional growth, acting in the presumptive apical cell, perhaps in response to auxin signalling (Aoyama *et al.* 2012).

16.7 How are the shoot apical meristems in the two generations related?

The question of whether, and if so, how, the evolution of multicellularity in the haploid gametophyte and diploid sporophyte are related can be rephrased as a question of whether the two generations are homologous or antithetic in origin (Bowman *et al.* 2016; Haig 2008). A homologous origin implies the ancestral land plant possessed isomorphic generations with the two generations subsequently morphologically diverging, while an antithetic origin implies the two generations were heteromorphic at their inception and had distinct evolutionary origins. Given that Embryophytes are nested within a grade of charophyte algae, and the most closely related extant algae possess a multicellular haploid generation and a single celled diploid generation, this might then suggest that the origins of the two Embryophyte generations differ, and that the origin of the alternation of generations is antithetic. This was the view of Bower when he considered the gametophyte generation to be older, with the sporophyte generation evolving by an intercalation of mitoses between gamete fusion and meiosis (Bower 1908). However, since current phylogenetic analyses suggest monophyletic bryophytes rather than a bryophyte grade, a homologous origin cannot be conclusively excluded. And while extant Embryophytes have distinctly heteromorphic alternations of generations, some Devonian Embryophytes possessed life cycles in which the two generations were more similar to one another than in any extant land plant (Kenrick 2018). While phylogenetic and fossil evidence are equivocal, developmental genetic analyses of shoot meristems in the two generations indicate that although they may share some common modules (e.g. auxin, APB, CLE signalling), some act differently in the two generations and other components crucial to sporophyte shoot meristems are not required for gametophyte shoot meristems (Bowman *et al.* 2019). Thus, although the shoot meristems in the two generations share some genetic components, it appears unlikely that there was a comprehensive co-option of a developmental regulatory network from one generation to the other as would be predicted if the generations had a homologous origin. If this is the case, there are two questions remain to be answered — how did complex multicellularity evolve in the gametophyte (assuming this is the older multicellular generation) and how much of the pre-existing gametophyte genetic machinery was co-opted during the evolution of complex multicellularity in the sporophyte generation.

16.8 Conclusion

Multicellularity arguably achieved its highest complexity in the Embryophyta and Metazoa lineages. Animal multicellularity can be traced back to a single unicellular lineage and is limited to the diploid phase of the life cycle. In contrast, in land plants complex multicellularity evolved in both the haploid and diploid generations. The haploid

multicellularity of land plants has likely antecedents in related charophyte algal lineages, whereas the evolution of a multicellular diploid generation occurred in the ancestral land plant, likely as an adaptive response to ephemeral conditions for aquatic fertilization in terrestrial habitats. Major questions remain about the relationship between the two complex multicellular generations, whether genetics programs of the presumed older multicellular haploid generation were co-opted to regulate aspects of the multicellular diploid generation, or whether the evolution of complex multicellularity in the two generations utilized largely independent genetic programs.

Acknowledgements

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