CHAPTER ONE

The liverwort *Marchantia polymorpha*, a model for all ages

John L. Bowman*
School of Biological Sciences, Monash University, Melbourne, VIC, Australia
*Corresponding author: e-mail address: john.bowman@monash.edu

Contents

Abstract

The liverwort *Marchantia polymorpha* has been known to man for millennia due to its inclusion Greek herbals. Perhaps due to its familiarity and association with growth in, often, man-made disturbed habitats, it was readily used to address fundamental biological questions of the day, including elucidation of land plant life cycles in the late 18th century, the formulation of cell theory early in the 19th century and the discovery of the alternation of generations in land plants in the mid-19th century. Subsequently, *Marchantia* was used as model in botany classes. With the arrival of the molecular era, its organellar genomes, the chloroplast and mitochondrial, were some of the first to be sequenced from any plant. In the past two decades, molecular genetic tools have been applied such that genes may be manipulated seemingly at will. Here, are past, present, and some views to the future of *Marchantia* as a model.

Land plants dominate nearly all terrestrial ecosystems and their origin dramatically changed Earth's geochemical cycles, as well as the evolutionary trajectories of many other lineages of life. Land plants evolved from an ancestral freshwater charophycean alga, with the extant lineage of land plants arising near the base of the Dapingian in the mid-Ordovocian, ca 470 MYA (e.g., Delwiche & Cooper, 2015; Edwards, Morris, Richardson, & Kenrick, 2014; Strother & Taylor, 2018). Perhaps unsurprisingly, due to

Current Topics in Developmental Biology, Volume 147
ISSN 0070-2153
https://doi.org/10.1016/bs.ctdb.2021.12.009

the open ecological niches available, land plants rapidly diversified, with all six extant land plant lineages (liverworts, mosses, hornworts, lycophytes, ferns, seed plants) established by the Middle Devonian (Morris et al., 2018). By the end of the Devonian, complex ecosystems with metazoan herbivory and fungal and bacterial detritivory similar to those of the present had evolved. From a botanical perspective, other than the lack of flowers and recognizable species, one might not have felt "out of time" walking through the Late Devonian forests with layered canopies and large trees. To reconstruct the nature of the ancestral land plant and its subsequent evolution, the interrelationships of extant land plant lineages and sister lineages of charophycean algae must be resolved and combined with morphological and anatomical attributes gleaned from extant and fossil plants. Given the pivotal nature of the origin of a land flora, it has been a subject of investigation and speculation for more than a century, but only recently have hypotheses converged on a scenario that is generally agreed upon. This is in large part due to the development of relevant genetic and/or genomic land plant and charophycean algal model systems in which evolutionary questions can be addressed.

The transition to a terrestrial habitat necessitated physiological adaptations to cope with abiotic stressors (e.g., desiccation and UV radiation) as well as developmental innovations to disperse desiccation tolerant spores. For example, at a fundamental level, at least two major changes in life cycle histories must have accompanied the origin and diversification of land plants. First, the sister charophycean algae have a haplontic life cycle with a multicellular haploid phase and a single-celled diploid zygote. In contrast, land plants have complex multicellular bodies in both their haploid and diploid phases, and the evolution of a multicellular diploid embryo gives land plants their taxonomic name, Embryophyta. This innovation allowed the production of a large number of spores from a single aquatic fertilization event, an often-limiting factor in terrestrial habitats. Second, extant land plants may have life cycles in which the haploid phase is dominant, with the diploid phase largely dependent and ephemeral, as in all extant bryophytes (liverworts, mosses, hornworts), or a life cycle in which the diploid phase is dominant and the haploid phase transitory, as in all extant vascular plants (lycophytes, ferns, seed plants). Due to their being the source of much (or all) of our food, fiber, and timber almost all land plant model systems are seed plants, with nearly all models being angiosperms. However, in recent decades it has become apparent that having model systems in each of the six lineages would be useful to address fundamental questions

concerning land plant evolution. Several recent reviews have focused on the liverwort *Marchantia polymorpha*, including ones covering anatomical details of the life cycle (Shimamura, 2016), the history of its use in experimental biology (Bowman, 2016), the development of genetic and genomic tools (Ishizaki et al., 2015; Ishizaki, Nishihama, Yamato, & Kohchi, 2016; Nishihama, Ishida, Urawa, Kamei, & Kohchi, 2016; Sauret-Güeto et al., 2020), nomenclatural issues (Bowman, Araki, et al., 2016), recent advances in understanding its physiology and development (Hisanaga et al., 2019; Kohchi, Yamato, Ishizaki, Yamaoka, & Nishihama, 2021; Romani & Moreno, 2021), and the coevolution of land plants with associated microbial symbionts and pathogens (Delaux & Schornack, 2021). In this review, I trace the history of using *Marchantia* as a model system and attempt to highlight both recent discoveries that have expanded our knowledge and areas in which additional investigation might be fruitful.

1. Why a liverwort and why *Marchantia*?

While *M. polymorpha*, hereafter *Marchantia*, has been utilized as a model system to investigate biological questions since the Enlightenment (discussed in more detail below), it's modern incarnation as model system dates to the past few decades. When cladistics began to be applied to the question of the origin and evolution of land plants, DNA sequence data was not widely available, and morphology and anatomy provided the bulk of the phylogenetically informative characters. On these criteria, liverworts were resolved as the first lineage of land plants to diverge from the others, largely on the basis of their lack of stomata, which are present is all other land plant lineages (e.g., Kenrick & Crane, 1997). Subsequently, when nucleic acid sequences became more plentiful, in many studies liverworts continued to be placed as the diverging from the most basal node within land plants (e.g., Qiu et al., 2006). It was with perspective in mind that two research groups, Takayuki Kohchi's (University of Kyoto) and my own (Monash University), proposed a *Marchantia* genome project to the US DOE Joint Genome Institute in 2007. During the time that this genome project took to reach fruition (published in 2017), our views of the relationships between the six land plant groups also evolved. Although nearly every possible topology has been proposed at some time, phylogenomic analyses have converged upon a consensus topology wherein the three bryophyte lineages are a clade and the three vascular plant lineages are a clade (e.g., de Sousa, Foster, Donoghue, Schneider, & Cox, 2019; Nishiyama et al., 2004; Puttick

et al., 2018; Wickett et al., 2014). While this topology is the consensus, it is only supported by about half of individual gene trees, perhaps reflecting the rapid divergence of the six lineages from one another, especially those of the three bryophytes (Fig. 1). In this respect, each of the six extant lineages has

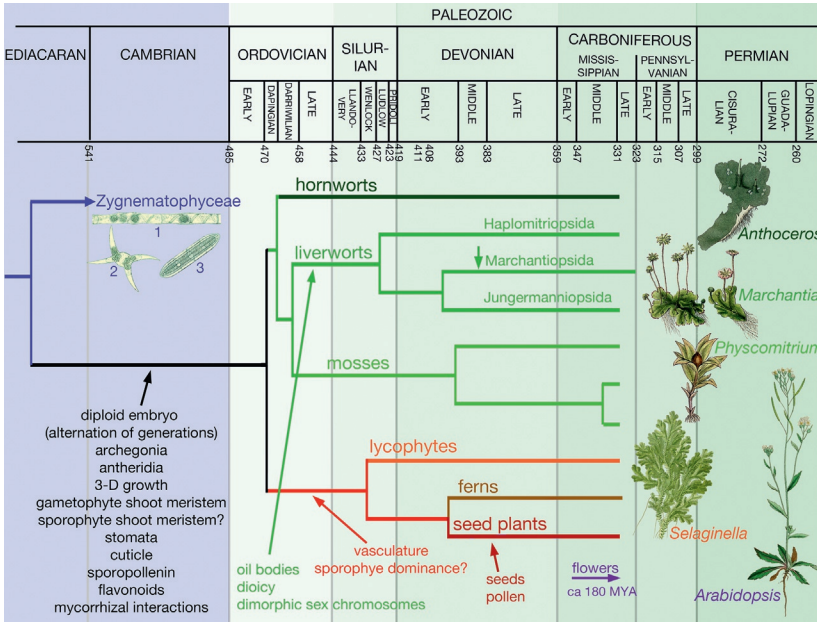

Fig. 1 The Phylogenetic context of *Marchantia*. The sister taxon to land plants is the Zygnematophyceae, a group that includes both multicellular filamentous forms, such as *Spirogyra* (1), and unicellular species, such as *Closterium* (2) and *Penium* (3). The six major extant lineages of land plants diverged by the middle Devonian, with the three lineages of bryophytes diverging from one another as early as the Ordovician. The three major extant lineages of both liverworts and mosses also diverged during this time frame. Representative models with genome sequence available of five land plant lineages are at the right—each of these is amenable to both forward and reverse genetic approaches, with the exception of *Selaginella* for which transgenic plants have not yet been reported. Key innovations during the evolution of land plants (*black*), liverworts (*green*), vascular plants (*red*), and seed plants (*dark red*) are listed, with allusions to many of these in the text. The green arrow on the Marchantiopsida lineage indicates the origin of derived characters, including complex thallus with air chambers, gametangiophores, and pegged rhizoids, found in *Marchantia*. Flowering plants, one of two seed plant lineages evolved later, with the blooming of the first flowers (*purple*) estimated to be around 180 MYA. The approximate divergence times of nodes in the trees were adapted from previous estimates of early land plant divergences (Morris et al., 2018), with the timing of the initial divergence between bryophytes and tracheophytes adjusted to 470 MYA corresponding to the origin of land plant cryptospore morphology (Strother & Taylor, 2018).

been on its own isolated evolutionary trajectory for the past 400+ million years (Bowman, 2013). If the currently accepted consensus topology is indeed a genuine reflection of evolutionary history, the liverworts do not occupy a unique position, but rather all bryophyte lineages are equally related to those of vascular plants, and as discussed elsewhere, resolution of monophyletic clades of bryophyte and vascular plants renders many ancestral attributes, including the nature of the life cycle, ambiguous (Bowman, Briginshaw, & Florent, 2019). Thus, in the reconstruction of ancestral character states, analyses should focus on comparisons amongst members of all six extant lineages.

The usefulness of a model system depends upon both its suitability for use in experimental (e.g., laboratory) studies and its unique combination of synapomorphies and derived characters that facilitate the investigation of particular biological questions (Fig. 1). While *Marchantia*'s modern incarnation as a model system is founded on its facile genetics and genomic simplicity, previous manifestations (Table 1) were likely primarily due to its familiarity as a colonizer of man-made disturbed habitats (Schuster, 1992). As such, *Marchantia* has been known to man for millennia, being one of the several hundred medicinal plants first described by Dioscorides and Pliny in the first century CE, and then continuously in herbals through to the Renaissance (Bowman, 2016). Following the Enlightenment, *Marchantia* was employed in studies to elucidate the bryophyte life cycle (Hedwig, 1783), and then with the advent of microscopy where spherical and chromatic aberrations were corrected, *Marchantia* was utilized for studies on cell theory (Mirbel, 1835), motility of bryophyte sperm (Unger, 1837), and Hofmeister's discovery of the alternation of generations, which unified land plants as a natural group (Hofmeister, 1862). During this time, related liverwort species were used as models to investigate the structure and function of shoot meristems, with the discovery that many shoot meristems possess a single apical cell from which the remainder of the plant body is derived (Nägeli, 1845).

The ubiquity of *Marchantia* led to its use in the classroom as a model for both botany and general microscopy from the mid 19th century onwards, with some enthusiastically endorsing liverworts to entice students: *But if you wish to elicit from your audience exclamations of delight, put on the slide a freshly ruptured spore-case of one of the larger Hepaticae*—Marchantia*,* Asterella*, or* Conocephalum *will answer. The lively way in which the spiral elaters toss the spores about is sure to "bring down the house"* (Blanchard, 1891). However, not all were enamored with the use *Marchantia* due to its derived features, and it was written in a review of Scott's botany textbook in Bohr, 1897:

Table 1 Landmarks in the history of *Marchantia*.

1st–17th centuries	Description of *Marchantia* as a treatment to prevent inflammation of wounds in Greek herbals	Bowman (2016)
1713	Jean Marchant provides a name	Marchant (1713)
1783	Hedwig's use to discover the bryophyte life cycle	Hedwig (1783)
1835	Mirbel's use in investigating cell theory	Mirbel (1835)
1837	Unger's discovery of motile sperm in bryophytes	Unger (1837)
1845	Nägeli's investigation of apical cells (in a related species)	Nägeli (1845)
1851	Hofmeister's elucidation of the alternation of generations	Hofmeister (1862)
1917	Allen's discovery of sex chromosomes in *Sphaerocarpos*	Allen (1917)
1928	Heitz's description of heterochromatin in *Pellia*	Heitz (1928)
1943	Burgeff's genetic studies of *Marchantia*	Burgeff (1943)
1986	*Marchantia paleacea* chloroplast genome sequence	Ohyama et al. (1986)
1992	*M. paleacea* mitochondrial genome sequence	Oda et al. (1992)
2007	JGI *Marchantia* genome project initiated	https://genome.jgi.doe.gov/portal/Marpha_FD/Marpha_FD.info.html
2008	Transformation via *Agrobacterium*	Ishizaki, Chiyoda, Yamato, and Kohchi (2008)
2013	Homologous recombination	Ishizaki, Johzuka-Hisatomi, Ishida, Iida, and Kohchi (2013)
2014	CRISPR/Cas9 genome editing	Sugano et al. (2014) and Sugano et al. (2018)
2016	Conditional knockouts	Nishihama et al. (2016)
2017	Nuclear genome sequence	Bowman et al. (2017)
2020	Chromosome based nuclear sequence	Montgomery et al. (2020)

"which its complex and highly specialised structure render it eminently unfit to occupy" (Bohr, 1897). Due to the influx and availability of plants sourced from all over the globe, this era encompassed the flourishing of gardening. *Marchantia* was noted in this context as well, but not in a good light, as it was described as a "troublesome weed" (Smee, 1872) and "the greatest enemy" (Robinson, 1910). However, even the gardeners occasionally marveled at its beauty, "*Although so troublesome a weed, it is welcome in small quantities, and is one of our finest natural plants*" (Smee, 1872).

With the advent of the chromosome theory of inheritance, the first plant sex chromosomes were discovered in a related liverwort, *Sphaerocarpos texanus* (Allen, 1917), and the first description of eukaryotic heterochromatin was made in the liverwort *Pellia epiphylla* (Heitz, 1928). Following Muller's demonstration that X-rays are mutagenic, *Marchantia* and related liverwort species were subjected to intensive genetic studies, many aimed at elucidating the biology of sex chromosomes (Bowman, 2016), with classical genetic experiments culminating in Burgeff's monograph on the genetics of *Marchantia* (Burgeff, 1943). In the molecular era, sequence of the complete chloroplast genome of *Marchantia paleacea* was the second determined for any plant species (Ohyama et al., 1986) and that of the *M. paleacea* mitochondrial genome was the first land plant sequence available (Oda et al., 1992). Note that these two genomes were originally published as those of *M. polymorpha*, but were later shown to be derived from *M. paleacea* cultures (Bowman et al., 2017). Thus, the use of *Marchantia* as a model system has paralleled advances in biological thought and technological advances over the past few centuries.

While most research over the past few decades on land plant biology has been concentrated on flowering plant species, there has been a recent resurgence of interest in phylogenetically diverse land plants to address issues of the origin and diversification of a land flora. For this, *Marchantia* is well suited as a model system due to its genetic and genomic attributes. Perhaps due to its "weedy" nature, *M. polymorpha* is easy to grow in the laboratory, with its life cycle completed in as little as 3 months. *M. polymorpha* is a dioicious species, i.e., with separate male and female plants, and as such crosses between lines are easily accomplished. As has been reviewed in more detail elsewhere (Ishizaki et al., 2016), the creation of transgenic plants via multiple methods (Ishizaki et al., 2008; Kubota, Ishizaki, Hosaka, & Kohchi, 2013; Tsuboyama, Nonaka, Exura, & Kodama, 2018), including insertion of multiple different transgenes simultaneously (Ishizaki et al., 2015), insertion of DNA via homologous recombination (Ishizaki, Johzuka-Hisatomi, et al., 2013), CRISPR-Cas9

mediated genome editing (Sugano et al., 2014; Sugano et al., 2018), and conditional alleles (Flores-Sandoval, Dierschke, Fisher, & Bowman, 2016; Nishihama et al., 2016), is routine, and in general, not limiting. Since the dominant generation is haploid, the time from the designing of guide RNAs for CRISPR-Cas9 genome editing to observing a mutant phenotype can be as little as two months, although subsequent genetic work would be required to confirm the genetic lesions. Thus, the creation of loss-of-function and gain-of-function alleles as well as reporter lines, either using histochemical or fluorescent proteins encoded by reporter genes, can be accomplished rapidly. Such ease of genetic manipulation facilitates construction of more sophisticated genetic combinations, such as conditional alleles to circumvent lethality of null alleles (Nishihama et al., 2016).

One advantageous feature that was foreshadowed by early work on specific gene families is the lack of whole genome duplications (WGDs) in *Marchantia*. This feature is characteristic of liverworts, which appear to lack ancient WGDs as a group. Thus, gene families, specifically those encoding regulatory proteins, have minimal numbers of paralogs, simplifying mutant studies. Further, as a consequence of the lack of WGDs, the regulatory genome of Marchantia may closely resemble that of the ancestral land plant (Bowman et al., 2017). It was postulated that the lack of ancient WGDs in Marchantia, and liverworts in general, was due to the early evolution of highly dimorphic sex chromosomes (Bowman et al., 2017; Iwasaki et al., 2021). Thus, the evolution of sex chromosomes may have facilitated preservation of ancestral genomic characteristics in this lineage. More than a half century ago Ohno proposed that a lack of WGDs might constrain subsequent evolution, which would then be canalized without a mechanism for large scale gene duplication (Ohno, 1970). Ohno suggested that the evolution of novelty would be more likely to arise from extant lineages that could undergo WGDs, as they would have the potential to possess a large number of paralogs that could subsequent neofunctionalize, leading to new evolutionary trajectories (Ohno, 1970). Given the lack of ancient WGDs in liverworts, it can be speculated that the gene regulatory networks of liverworts have been evolutionarily constrained, and thus may more closely resemble ancestral gene regulatory networks. Whether morphological evolution within the liverwort lineage has been similarly canalized is an open question.

The remainder of this review is an outline of the *Marchantia* life cycle, in which is embedded a discussion of synapomorphies and derived features (i.e., features and limitations), that make *Marchantia* a model system for

the investigation of various biological processes. Only references to the original discoveries and studies in *Marchantia* in the post-genomic era are highlighted, and for reasons of space limitations, this review is focused on developmental and evolutionary questions. In addition, a non-exhaustive set of specific questions of interest, from the authors perspective, are in italics throughout the text.

2. The sexual life cycle

A detailed description of the modern view of the *Marchantia* life cycle is presented in Shimamura (2016), however, many morphological and anatomical details were worked out prior to the turn of the 20th century (Fig. 2). Some older references are highlighted here along with relevant ones subsequent to Shimamura (2016).

2.1 The haploid gametophyte generation

Dispersed haploid spores that initiate the gametophyte generation (**1**) will germinate in the presence of light and moisture, the first sign being a swelling of the spore (**2**) with rupture of the exospore preceding accumulation of chlorophyll (Leitgeb, 1877a; O'Hanlon, 1926). Addition of exogenous auxin, a phytohormone, can induce germination in the dark, in essence substituting for the light stimulus (Fitting, 1939). Auxin is arguably the most important phytohormone, and transcriptional changes downstream of auxin signaling affect nearly all of plant development, prominently including tropic responses to gravity and light. *Marchantia* has been instrumental as a model system to reveal that auxin transcriptional signaling evolved in the ancestral land plant and, due to the lack of genetic redundancy of auxin signaling components in *Marchantia,* it was clarified that auxin acts as a facilitator, rather than a determiner, of developmental decisions (Bowman et al., 2017; Flores-Sandoval et al., 2018; Flores-Sandoval, Eklund, & Bowman, 2015; Kato et al., 2015; Kato et al., 2017; Kato et al., 2020). This topic has recently been reviewed (Bowman, Flores-Sandoval, & Kato, 2021; Suzuki, Kohchi, & Nishihama, 2021).

The first division of the germinating spore is usually asymmetric (**3**) producing a basal cell that will differentiate into a rhizoid and an apical cell that will give rise to the sporeling shoot (Mirbel, 1835; O'Hanlon, 1926). *The genetic and physiological bases of this initial asymmetric division are not known*, although auxin (see above) is likely to play a role. The apical region of the sporeling continues to proliferate producing a mass of cells (**4**), and

Fig. 2 The sexual life cycle of *M. polymorpha*. In the green shaded region is the haploid gametophyte and in the blue region is the diploid sporophyte, with spore dispersal in aqua. Individual numbered stages described in the text. Drawings from Groenland (1854); Hedwig, 1783), Henfrey (1853), Hofmeister (1862), Leitgeb (1881), Marchant (1713), Mirbel (1835), Mottier (1891), Schwann and Schleiden (1847), Thuret (1851), and Unger (1837).

subsequently a single apical cell is specified in a region corresponding to the highest incident light (Hansel, 1876; Leitgeb, 1877a). The specification of an apical cell defines the prothallus (**5**), with the apical cell having two division planes such that a heart-shaped unistratose structure develops (Bischoff, 1835; Leitgeb, 1881; Menge, 1930). *The molecular mechanism by which a single*

cell is chosen to be the apical cell is unknown, but again, light and likely auxin are both critical, as loss of auxin biosynthesis results either in a failure to specify or maintain an apical cell (Eklund et al., 2015).

Under optimal growth conditions the prothallus rapidly transitions into a complex thallus (characteristic of, and specific to, the class Marchantiopsida of liverworts), with dorsal air chambers and ventral scales and rhizoids, which will be described later. However, under suboptimal low light conditions, it may revert back to a filamentous protonemal growth until it reaches a more suitable light environment (Leitgeb, 1877a; Schostakowitsch, 1894). A similar extension of filamentous sporeling growth is observed when grown under red light (Téodoresco, 1929). During the transition from prothallus to complex thallus, the apical cell also transitions from possessing two cutting faces to four cleavage planes: dorsal, ventral and two lateral faces (Leitgeb, 1881; Mottier, 1891). *How the transition from 2-dimensional to 3-dimensional growth is regulated is unknown*, but it too is facilitated by auxin signaling since disruption of auxin signaling can results in plants failing to make the transition (Flores-Sandoval et al., 2015). While the precise pattern has not been observed in *Marchantia*, in *Blasia* a regular spiral pattern (lateral to dorsal to lateral to ventral) occurs (Leitgeb, 1874), and this likely to be the case for *Marchantia* (Apostolakos, Galatis, & Mitrakos, 1982) In longitudinal sections (**6**) the apical cell is conspicuous, but in transverse section, the apical cell is difficult to distinguish from immediate lateral derivatives (**7**), sometimes referred to as subapical cells (Leitgeb, 1879; Leitgeb, 1881; Mottier, 1891). However, both on anatomical and theoretical grounds, each apex harbors only a single apical cell (Douin, 1921; Mottier, 1891). While branching has not been directly observed due to lack of appropriate markers, it is likely to be pseudo-dichotomous, as has been observed in Jungermanniopsida simple thalloid and leafy liverworts (Kny, 1865; Leitgeb, 1877b), wherein the original apical cell is retained and a new apical cell is specified from a recent lateral derivative a cell or two distant from the original apical cell. *The molecular mechanisms of (pseudo)dichotomous branching are unknown*, however reduction in auxin levels can reduce branching (Eklund et al., 2015). Further, the land plant specific CLE peptide signaling systems modulate meristem function. Two distinct CLE peptide signaling systems, CLV3 and TDIF, existed in the ancestral land plant. In Marchantia, MpCLE1, a TDIF-type, restricts the proliferative activity of the gametophyte shoot meristem, in contrast to the stem cell promoting activity of TDIF in angiosperm vascular meristems (Hirakawa et al., 2019). Conversely, MpCLE2, a CLV3-type, promotes the accumulation of stem cells, likely apical cell derivatives, within the *Marchantia* gametophyte shoot

meristem (Hirakawa et al., 2020). For instance, application of exogenous CLE2 peptide can cause an excessive accumulation of stem cells with the potential to become multiple apical cells, and conversely, loss-of-function alleles have a reduced number of meristematic cells (Hirakawa et al., 2020). *The regular periodic dichotomous branching of the vegetative thallus suggests a broader intercellular signaling network, perhaps involving an interaction between the CLE and auxin signaling pathways and as yet unknown other factors.* That the orthologous CLE signaling systems operate in angiosperm sporophyte meristems in the opposite manner, suggests that while gametophyte and sporophyte shoot meristems share some genetic components, their genetic wiring may be substantially different. *The relationship between the shoot meristems of the gametophyte and sporophyte generations raises the question as to whether the two generations are homologous,* i.e., *utilizing a shared ancestral genetic program, or antithetic, whereby they would have two distinct evolutionary origins and trajectories.*

Similar to the predicted ancestral liverwort, *Marchantia* is dioecious with sex determined chromosomally (see Bowman, 2016, for review of sex chromosomes). Sex chromosomes in plants were first discovered in a related liverwort, *S. texanus* (Allen, 1917), and it has been suggested that the ancestral liverwort possessed sex chromosomes (Berrie, 1963). Two of the four meiotic products inherit a U (X in the older literature) chromosome and develop as female (**8**), while the other two meiotic products inherit a V (Y) chromosome and develop as males (**9**). In phylogenetically divergent liverworts (*Marchantia*, *Sphaerocarpos*, *Pellia*) genetic experiments have implicated a U chromosome feminizer locus that is sufficient to specify female sex (Haupt, 1932; Knapp, 1935; Lorbeer, 1936; Heitz, 1949–1951). *Marchantia* has proven an excellent model for the investigation of sex determination and the evolution of sex chromosomes (Bowman et al., 2017; Montgomery et al., 2020; Yamato et al., 2007). In *M. polymorpha* the feminizer locus encodes a member of the plant-specific BASIC PENTACYSTEINE transcription factor family, Mp*BPCU* (Iwasaki et al., 2021). Surprisingly, Mp*BPCU* has a homolog (gametolog) on the V chromosome, Mp*BPCV*, and both genes act in the reproductive transition but only BPCU possesses a function in sex determination (Iwasaki et al., 2021). MpBPCU negatively regulates SUPRESSOR OF FEMINIZATION, a cis acting antisense non-coding RNA, that in turn negatively regulates MpFGMYB, an autosomal gene encoding a land plant specific MYB transcription factor that promotes feminization (Hisanaga et al., 2019; Iwasaki et al., 2021). Arabidopsis FGMYB orthologs were also shown to promote female gametophyte development (Hisanaga, Okahashi, et al., 2019),

providing an example of discoveries made in *Marchantia* informing investigations in other more intensively studies taxa. *Whether the molecular identity of the feminizer is conserved throughout liverworts and beyond or whether there has been "turnover" of the sex determining gene are open questions.*

Gametangia are borne upon gametangiophores, which are specialized extensions of the dorsal-ventral thallus that grow orthotropically to facilitate gamete and spore dispersal (Bischoff, 1835; Hofmeister, 1862; Mirbel, 1835; Sachs, 1882). Gametangiophores evolved early in the Marchantiopsida lineage following the divergence of the Blasiales, and were subsequently lost in several taxa (Goebel, 1930). *Marchantia* is a long day plant (Benson-Evans, 1961; Wann, 1925), making the transition to reproduction in the spring in response to changes in light quality, i.e., red to far-red ratios (Courtoy, 1964), such that mature sporophytes are usually conspicuous in early summer in temperate climates (e.g., Bock, 1552). Experiments in *Marchantia* have demonstrated that the molecular machinery regulating the day-length dependent reproductive transition is at least partially shared across land plants (Kubota et al., 2014). For example, the single phytochrome protein, MpPHY, encoded in the genome acts as a red and far-red light receptor whose action is mediated via the downstream transcription factor MpPIF (Inoue et al., 2016). MpPHY is required for the reproduction transition, with loss-of-function alleles failing to initiate anatomical or morphological changes associated with the reproductive transition (Inoue, Nishihama, Araki, & Kohchi, 2019). Similarly, circadian clock genes conserved as well (Linde et al., 2017), with one output of the clock being rhythmic growth, or nyctinasty (Lagerkrantz, Billhardt, Rousku, Ljung, & Eklund, 2020).

During the reproductive transition, gametangiophores develop from one of two apices (**10**) produced from the most recent thallus bifurcation (Leitgeb, 1881). In *Marchantia* the gametangiophore capitula are the product of three (usually) rapid bifurcations forming eight meristems (Leitgeb, 1880b), each of which produce a series of gametangia, either archegonia or antheridia, from dorsal epidermal derivatives (**11**, **12**) (Hofmeister, 1862). These rapid bifurcations are then elevated via elongation of modified thallus growth, forming the gametangiophore which serves to elevate both the gametangia pre-fertilization and the sporophyte post-fertilization. The activity of a regulatory module involving a land plant-specific transcription factor, MpSPL2 and microRNA156/529 is required for proper gametangiophore development (Tsuzuki et al., 2019). Furthermore, the transition to reproductive growth and the specification of both male and female generative cells in *Marchantia* is dependent upon a land plant-specific bHLH

transcription factor, MpBONOBO, which is expressed in archegonial and antheridial initial cells (Yamaoka et al., 2004; Yamaoka et al., 2018). BONOBO orthologs also have a role in generative cell specification in Arabidopsis male gametophytes (Yamaoka et al., 2018), highlighting the conservation of molecular mechanisms of reproductive development across land plants. *The molecular mechanisms by which environmental signals are transduced to activate BONOBO, and furthermore in only one apex of a recently bifurcated shoot, remain unknown*. Archegonia and antheridia are innovations of the ancestral land plant (Fig. 1) and the two structures are thought to be homologous (Goebel, 1902). *While no archegonia or antheridia specifying gene has yet been identified, the two structures might be specified by the activity of BNB and FGMYB, with BNB alone conferring antheridial identity and the combination of both conferring archegonial identity*.

Archegonia harboring a single egg cell ensheathed in unistratose archegonium wall (Hofmeister, 1862; Mirbel, 1835; Strasburger, 1869) (**13**) are produced in a row with the oldest towards the outside of the archegoniophores (**11**) (Bischoff, 1835; Mirbel, 1835) due to epinastic growth of the apex displacing the apical cell ventrally (Hofmeister, 1862; Leitgeb, 1881). Egg cells are, at least in part, specified by the activity of an RKD transcription factor, MpRKD, and in its absence, the cell that would normally differentiate into an egg cell does not arrest and instead continues to divide (Koi et al., 2016; Rövekamp, Bowman, & Grossniklaus, 2016). Likewise, antheridia (**14**) form in irregular rows, but with the oldest towards the center of the capitulum (**12**) (Hedwig, 1783; Schmidel, 1762). Sperm mother cells are formed by synchronized cells divisions within sectors of the antheridia (**14**). Motile sperm with two anterior flagella (**15**) (Unger, 1837) are extruded through antheridial pores due to swelling of the mucilage containing antheridial jacket cells when in contact with water (Bergdolt, 1926; Goebel, 1898; Strasburger, 1869). Sperm differentiation is regulated in part by the MYB transcription factor MpDUO (Higo et al., 2018), whose Arabidopsis ortholog also regulates sperm differentiation. The role of DUO in regulating sperm morphogenesis likely predates land plants, with its origin in an ancestral charophyte alga at the time that anisogamy evolved in the Streptophyte lineage (Higo et al., 2018). *The mechanism by which male gametes are specified remains unknown, as does the mechanisms by which MpRKD is activated in the egg cell*.

Fertilization is aquatic, but it is likely sperm swim only a short distance, with other mechanisms facilitating long distance events. Males produce gametangiophores earlier than females, and those of males elongate as

antheridia are maturing, while those of females harbor mature egg cells before substantial elongation has occurred. In some Marchantiopsida sperm are forcibly ejected (Cavers, 1903; Peirce, 1902; Thuret, 1851), but not in *Marchantia*. However, the elevated antheridiophore forms a splash platform from which a limited amount of sperm may be dispersed short distances (Duckett & Pressel, 2009; Strasburger, 1869). However, it is not free spermatozoid cells that are ejected, but rather sperm that are still contained in their mother cell membrane (Strasburger, 1869), which allows the sperm to be transported via spread of a lipophilic layer on water surfaces (Muggoch & Walton, 1942). Thus, the 200,000+ sperm produced per antheridia (Bolleter, 1905; Johnson, 1904) could spread tens of meters from the source (Pressel & Duckett, 2019). The scales and rhizoids on the ventral thallus surface, as well as in the archegoniophore rhizoid furrow, allow rapid transport via capillary action (Duckett, Ligrone, Renzaglia, & Silvia, 2014; McConaha, 1941) to the top of the archegoniophores, such that the sperm may only have to swim >1 mm (Shimamura, 2016). Thus, in a natural setting, fertilization was observed to occur between male and female plants located almost 20 m apart (Pressel & Duckett, 2019). While the mucilage extruded by the archegonia is thought to provide chemotactic cues for sperm (Strasburger, 1869), *the precise nature of the chemicals involved is unknown* despite some chemicals acting as chemo-attractants in vitro (Åkerman, 1910; Lidforss, 1904–5).

2.2 The diploid sporophyte generation

Following fertilization, the diploid zygote representing the sporophyte generation expands to fill the venter (**16**) (Hofmeister, 1862). The genetic mechanism by which the diploid sporophyte gene expression program is activated likely involves paralogous homeodomain genes, as such a system is active diverse eukaryotes and likely represents a character of the ancestral eukaryote (Bowman, Sakakibara, Furumizu and Dierschke, 2016). In extant unicellular Viridiplantae, two paralogous TALE-homeodomain genes have mutually exclusive gamete specific expression patterns (Lee, Lin, Joo, & Goodenough, 2008). However, such systems may be more complex in multicellular lineages as there exists evidence that one, e.g., in the moss *Physcomitrium* (Horst et al., 2016; Sakakibara et al., 2013; Sakakibara, Nishiyama, Deguchi, & Hasebe, 2008), or both, e.g., in the brown alga *Ectocarpus* (Arun et al., 2019), TALE-homeodomain paralogs are expressed in both types of gametes. Indeed, in *M. polymorpha* one class of

TALE-homeodomain paralog, Mp*KNOX1*, is expressed in the egg cell and is absolutely required for activation of the diploid sporophyte program, while genes of the other class of TALE-homeodomain gene, Mp*BELL3* and Mp*BELL4*, are expressed in both the the egg cell and developing sperm (Dierschke et al., 2021; Hisanaga et al., 2021). Loss of either male or female BELL greatly reduces efficient of activation of the diploid program and loss in both parents resembles loss of Mp*KNOX1* (Dierschke et al., 2021; Hisanaga et al., 2021). *How the diploid sporophyte program is specifically activated post-fertilization in systems in which one or both TALE-homeodomain paralogs are expressed in both parents is unknown.*

The first division of the zygote is usually transverse, with the epibasal cell (the one closer to the archegonial neck) eventually giving rise to the capsule and the hypobasal cell (the one closer to the archegonial stalk) giving rise to the foot and short seta (Leclerc du Sablon, 1885). The next divisions are typically vertical resulting in a "quadrant" embryo (**17**). There is no conspicuous apical meristem, but rather, cell divisions are dispersed through the embryo (Kienitz-Gerloff, 1874), and after a couple weeks differences are evident between the cells of the foot and seta compared to the cells that will give rise to the capsule (Durand, 1908; Kienitz-Gerloff, 1874) (**18**). *Little is known about the genetic basis of patterning events during early sporophyte development,* i.e., *how the foot, seta and capsule are specified.* During the early growth of the embryo, both periclinal and anticlinal divisions in the gametophytic calyptra occur (**17**) such that its growth keeps pace with that of the ensheathed sporophyte (**19**) (Hofmeister, 1862). The developing sporophyte is also surrounded by a gametophytic pseudoperinath that develops post-fertilization and groups of archegonia derived from the same apical cell are surrounded by an involucre, such that three gametophytic tissues surround developing sporophytes (Mirbel, 1835). *The biochemical nature of communication pathways between the two generations remains to be explored.*

Subsequently, internal cells of the capsule begin to elongate in the vertical plane (**20**) and a subset of these cells, elaterocytes, are set aside to differentiate into diploid elaters (**21**) while the other cells are sporogenous and divide transversely to produce sporocytes (**22**) (Henfrey, 1853). In *Marchantia*, the sporogenous cells typically divide five times producing 32 sporocytes, each of which undergoes meiosis generating four haploid spores (Henfrey, 1853) (**23**), for a total of 128 spores per elater (O'Hanlon, 1926). Each sporophyte can generate approximately 300,000 spores (O'Hanlon, 1926). *Little is understood about the specification of elaterocytes* versus *sporogenous cells, or of the regulation of the number of divisions of the latter before their*

specification as meiocytes. The foot is the conduit between the maternal gametophyte generation and the diploid sporophyte generation, providing both nutrients and water. While there are no plasmodesmatal connections between generations (Kelley, 1969), extensive ingrowths occur in presumed transfer cells of both generations (Ligrone, Duckett, & Renzaglia, 1993). *The signaling networks that must be involved in establishment of communication and nourishment channels between the generations are as yet unexplored. Further, whether there is any genomic imprinting associated with the dependency of the embryo on the female gametophyte is unknown*. Prior to capsule rupture the seta elongates, as in other liverworts, via cell elongation (Thomas, 1980), with the relatively short seta of *Marchantia* being a derived condition since the gametangiophores serve to elevate the sporophytes to facilitate spore dispersal.

The unistratose capsule wall ruptures (**20**, **24**) aided by annular cell wall thickenings, similar to other liverworts (Leclerc du Sablon, 1885), and hygroscopic elaters, which are dead at maturity, facilitate spore dispersal (**25**, **26**) (Marchant, 1713). *The identity of the presumed carbohydrate polymers responsible for the hygroscopic movements of the elaters are not yet identified*. Under natural conditions, spore longevity in *M. polymorpha* is limited, with viability dropping substantially between 12 and 18 months post dispersal (Inoue, 1960; O'Hanlon, 1926). The smooth spores coated in sporopollenin, the most durable biological polymer known, can be dispersed widely enabling the colonization of distant disturbed, often recently burnt, habitats (e.g., Skutch, 1929; Torrey, 1932). *The mechanism of sporopollenin deposition is enigmatic as a tapetum is lacking in liverworts*. Spores have been detected in rainwater (Pettersson, 1940) and transoceanic dispersals have occurred within the genus as evidenced by colonization of oceanic islands (Bischler-Causse, 1989; Young & Kläy, 1971).

2.3 The vegetative thallus of the gametophyte

M. polymorpha is a complex thalloid liverwort with the vegetative gametophyte having a distinct dorsal-ventral structure (Fig. 3). The dorsal side (**1**) is characterized by a latticework of air chambers, while the ventral side (**2**) is covered with a patterned network of scales and rhizoids. Air chambers develop close to the apical mersitem from the dorsal epidermal and epidermal cell layers (Barnes & Land, 1907) (**3**) with each chamber having a centrally located multistratose air pore (Nägeli, 1842) (**4**, **5**) and basal photosynthetic assimilatory filaments (**5**). The extent of air chamber development is influenced upon light quality and intensity (Stahl, 1882), moisture

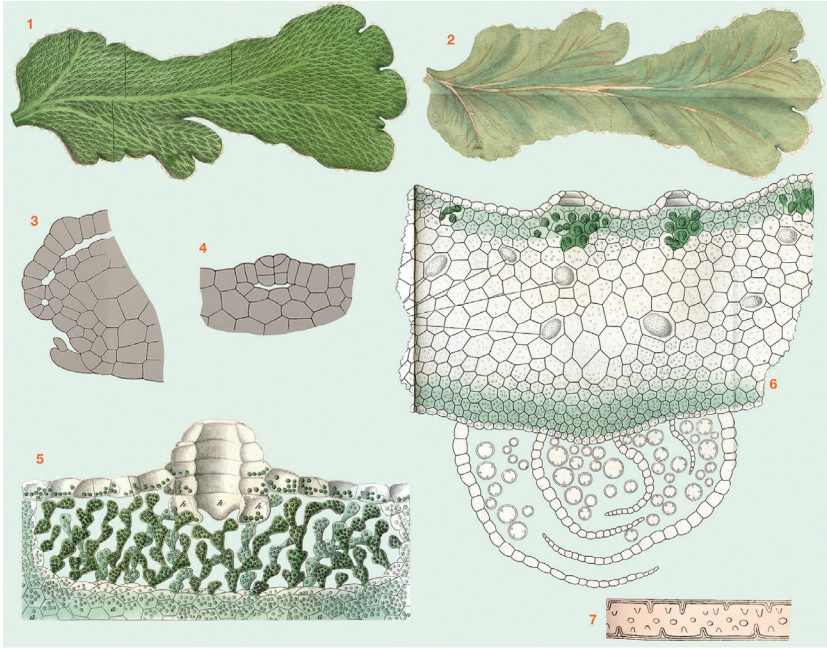

Fig. 3 *M. polymorpha* vegetative thallus anatomy. Dorsal (1) and ventral (2) overviews of vegetative thalli. Development of air chambers and pores (3, 4) and a fully developed air chamber (5). Cross section through mature vegetative thallus (6); unistratose scales arise in 2–4 rows flanking the ventral midrib, enveloping bundles of pegged rhizoids (6, 7). Smooth rhizoids arise from near the ventral midrib, but are not shown in this view. Fours lines at left highlight oil body cells (6). Individual numbered images described in the text. Drawings from Leitgeb (1880a), Mirbel (1835), and Nägeli (1842).

(Ruge, 1893), and CO2 concentration (Téodoresco, 1899). The architecture of air chambers does not correspond to merophytes, implying a requirement of cell-cell communication to coordinate their development (Suzuki, Harrison, Shimamura, Kohchi, & Nishihama, 2020). The E3 ubiquitin ligase NOPPERABO1 is required for the initial stage of formation of the intercellular spaces, with mutants lacking both air chambers and pores (Ishizaki et al., 2013). *The intercellular communication pathways responsible for the stereotypic pattern of air chambers and pores is not known.* While mostly inconspicuous elsewhere, the deposition of a cuticular layer is prominent on the air pore and surrounding cells (Schönherr & Ziegler, 1975). As in flowering plants, a land plant specific MYB transcription factor, MpMIXTA, regulates cuticle biosynthesis genes in *Marchantia* (Xu et al., 2021).

Ventral parenchyma acts as a storage tissue, and is interspersed with oil body cells, a plesiomorphic character of liverworts (**6**). While many roles have been postulated in the past, in *Marchantia* they do not play a role in abiotic stress responses, but rather provide protection against herbivores (Kanazawa et al., 2020; Romani et al., 2020), as postulated more than a century previously (Stahl, 1888). As predicted from earlier studies that placed terpene synthases at the oil body membrane (Suire et al., 2000), the oil bodies of *Marchantia* are filled with sequiterpenes and bis-bibenzyl compounds (Romani et al., 2020; Takizawa et al., 2021; Tanaka et al., 2016). Two unrelated transcription factors, MpERF13 and MpC1HDZ are required for oil body formation (Kanazawa et al., 2020; Romani et al., 2020), and the involvement of a SNARE protein, MpSYP12B, suggests that oil body formation within a cell involves a cell biological process similar to a typical plant cell division, i.e., that the oil body membrane is analogous to the plasma membrane lipid bilayer (Kanazawa et al., 2020).

From the ventral epidermis are formed 3–4 pairs scales in rows flanking the midrib (Taylor, 1837) (**6**). The proper development of the scales requires the transcription factor MpLOS1, which is predominantly expressed in scales and differentiating ventral tissues but also affects shoot meristem activity non-autonomously (Naramoto et al., 2019). The ventral scales, as well as the ventral epidermis are often pigmented red to purple (Nagai, 1915), which is thought to provide protection from UVB radiation. The pigment, a cell wall-bound auronidin, was recently shown to be formed via a phenylpropanoid biosynthetic pathway distinct from that leading to anthocyanins, the primary red pigments in flowering plants (Berland et al., 2019). However, the auronidin biosynthetic pathway is regulated via MpMYB14, an ortholog of land plant specific MYB genes that regulate anthocyanain biosynthesis in angiosperms (Albert et al., 2018; Kubo et al., 2018). Mp*MYB14* gain-of-function mutants that have increased flavonoid production have increased UVB tolerance, with UVR8-mediated induction in response to increased UVB conserved across land plants (Clayton et al., 2018).

Two types of rhizoids are produced, smooth and pegged (Gasparrini, 1856), and both consist of single cells that can be millimeters in length. The large diameter smooth rhizoids are negatively phototropic, tip growing, and emanate from near the midrib and act in anchorage and nutrient absorption (Haberlandt, 1889; Molisch, 1884; Pfeffer, 1871). The identification of a common conserved module involving bHLH transcription factors (MpRSL1 in *Marchantia*) regulating genes encoding proteins involved in tip growth revealed molecular conservation between *Marchantia* rhizoid

and Arabidopsis root hair development (Honkanen et al., 2016; Proust et al., 2016). Although *M. polymorpha* does not exhibit interactions with mycorrhizal fungi, in related species, such as *M. paleacea*, the smooth rhizoids provide an avenue of entry for mycorrhizal fungi (Radhakrishnan et al., 2020). The ability to interact with mycorrhizal fungi is ancestral, and is thought to have been crucial during the early terrestrial evolution of land plants (Rimington, Duckett, Field, Bidartondo, & Pressel, 2020; Strullu-Derrien, Selosse, Kenrick, & Martin, 2018). While the signaling pathway genes in *M. polymorpha* are reduced to pseudogenes, *M. paleacea* provides an excellent model to study mycorrhizal interactions by harnessing the genomic tools and technologies developed for *M. polymorpha* (Radhakrishnan et al., 2020).

In contrast to the smooth rhizoids, the pegged rhizoids (**7**) are dead at maturity (Cavers, 1904; Kamerling, 1897), and act in conjunction with the scales in capillary water transfer across the ventral surface of the thallus (Bowen, 1935; Duckett et al., 2014; McConaha, 1941). *Little is known about whether these cells undergo a typical programmed cell death program, or whether their cell wall thickenings are regulated in a similar manner as those of vascular cells that also undergo programmed cell death in vascular plants. As of yet, no mutant has been reported that differentially affects smooth or pegged rhizoids.*

2.4 Asexual reproduction

M. polymorpha can propagate asexually via the production of gemmae, which are produced in gemmae cups, which in turn are produced from the dorsal surface of the vegetative thallus (Fig. 4). Gemmae cup formation requires the function of the MYB transcription factor GCAM1 (Yasui et al., 2019) and the cytokinin signaling pathway (Aki et al., 2019; Flores-Sandoval et al., 2016). Gemmae develop from dorsal epidermal cells (Barnes & Land, 1908) just distal the apical meristem (**1**), initially via tip growth from single cells in the cup base (Hofmeister, 1862) (**2**). That each gemma is derived from a single epidermal cell allows the use of gemmae in "purifying" mutations generated by CRISPR-Cas9 genome editing occurring during sporeling development. Gemma initiation requires Mp*RSL1*, perhaps due to both rhizoids and gemma having their origin in outgrowths from single epidermal cells (Proust et al., 2016). Likewise, mucilage cells have a similar origin and also require Mp*RSL1* (Proust et al., 2016). In contrast, gemma initiation requires the ROP-GEF, KARRAPO, while neither rhizoids nor mucilage cells requires its function (Hiwatashi et al., 2019).

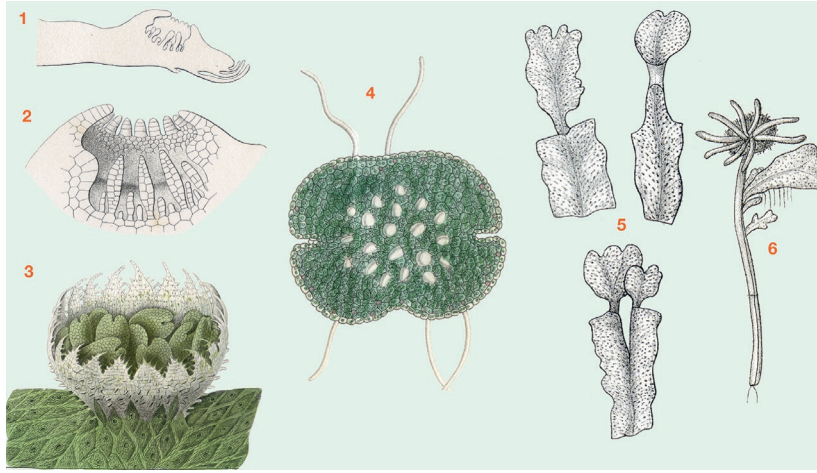

Fig. 4 Asexual reproduction in *M. polymorpha*. Developing gemmae cups and gemmae (1, 2). Mature gemmae cup with gemmae (3). Mature gemma (4); the central hyaline cells are rhizoid precursor cells, which may differentiate into rhizioids depending upon light and gravity cures. Regeneration can occur from almost all cells, except rhizoids; regeneration from vegetative thallus from which the shoot apex was removed (5), and from an excised archegoniophore (6). Individual numbered images described in the text. Drawings from Hofmeister (1862), and Mirbel (1835), and Vöchting (1885).

Hofmeister suggested that gemmae are not oriented at random, but have their surfaces perpendicular to the longitudinal axis of thallus (Hofmeister, 1862), suggestive of a polar signal emanating from the thallus, with the obvious candidate being auxin, which has been postulated to be transported from the apex into older tissues (Eklund et al., 2015; LaRue & Narayanaswami, 1957; Maravolo, 1976). Auxin transcriptional signaling is also important during pattern formation of developing gemmae, with its disruption leading to altered patterns of cell division planes and supernumerary shoot meristems (Kato et al., 2017).

Mature gemmae cups (**3**) act as splash cups with raindrops displacing the uppermost gemmae away from the parental plant (Brodie, 1951). For nearly the past two centuries the irreversible dorsiventrality induced in the initially "two-sided" lenticular gemmae (**4**) by light (primarily) and gravity has been investigated [see (Bowman, 2016) for review]. While gemmae reside in the cup they remain dormant, only resuming growth once displaced, with early experiments suggesting signals from outside the cup as well as within are required to impose and maintain dormancy (Molisch, 1922; Oppenheimer, 1922; Tarén, 1958). Genetic approaches have revealed that at least three

phytohormones participate in gemmae dormancy — an auxin signal, perhaps derived from the apical meristem and more locally at the base of the cup (Eklund et al., 2015), and a signal involving abscisic acid, a phytohormone known to be involved in dormancy throughout land plants, acting within the gemmae in the cup (Eklund et al., 2018) are both required for dormancy. Finally, the ethylene signaling pathway may provide an intermediary between these other two signals (Ruge, 1893; Eklund et al., 2018). One the first anatomical changes during gemmae germination is the growth of rhizoids from rhizoid initial cells, which are dispersed on both sides of mature gemmae (**4**). While the transcription factors promoting rhizoid and root hair growth are orthologous, the generation of a dispersed pattern produced by lateral inhibition is via independently evolved molecules, with a microRNA performing this function in *Marchantia*, as opposed to a protein based system in root hair patterning (Honkanen, Thamm, Arteaga-Vazquez, & Dolan, 2018; Thamm, Saunders, & Dolan, 2020).

As in many other bryophytes, *M. polymorpha* exhibits a high capacity for regeneration from gametophytic tissues from which the shoot meristem has been removed (**5**, **6**) (Dickson, 1932; Kreh, 1909; Nagai, 1919; Vöchting, 1885). In *Marchantia*, light, primarily via perception by phytochrome of red light, promotes regeneration, with the process greatly reduced under far-red light or dark conditions (Nishihama et al., 2015). Consistent with auxin having a role in regeneration, an ARF transcription factor, MpARF3, that may negatively regulate auxin signaling in some capacity, is required for efficient regeneration (Flores-Sandoval et al., 2018). Indeed, a reduction is auxin signaling is required for induction of a land plant-specific AP2 transcription factor, MpLAXR, that subsequently promotes regeneration (Ishida et al., 2022). *The processes of shoot regeneration and apical cell specification in the shoot meristem share genetic programs and studies on each should inform the other.*

3. The future

In order to ascertain the ancestral or derived nature of characters it is imperative to develop model systems amenable to both forward and reverse genetics in as many of the six major lineages as feasible. Such models could help overcome a major bias in current analyses by establishing a broader baseline for comparisons, rather than assuming the character states, often derived, in angiosperms as the standard. While past incarnations of *Marchantia* as a model system were primarily due to its familiarity, the present manifestation is based on a combination of phylogenetic relationships, facile

genetics and a regulatory genome that exhibits a paucity of genetic redundancy. This latter feature makes *Marchantia* well suited to large-scale experiments aimed at the identification of putatively ancestral land plant gene regulatory networks, as a baseline to determine how they have been modified and elaborated over evolutionary time. With its amenability to high throughput genetic manipulation and genomic editing *Marchantia* will be one of the languages of the Rosetta stone of land plants for some time to come.

Acknowledgments

This review was improved by the constructive comments of Facundo Romani and members of my lab. Research in J.L.B.s laboratory is supported by the Australian Research Council (DP200100225; DP210101423).

References

Åkerman, Å. (1910). Über die chemotaxis der *Marchantia*-spermatozoiden. *Zeitschrift für Botanik*, *2*, 94–103.

Aki, S. S., Mikami, T., Naramoto, S., Nishihama, R., Ishizaki, K., et al. (2019). Cytokinin signaling is essential for organ formation in *Marchantia polymorpha*. *Plant & Cell Physiology*, *60*, 1842–1854.

Albert, N. W., Thrimawithana, A. H., McGhie, T. K., Clayton, W. A., Deroles, S. C., et al. (2018). Genetic analysis of the liverwort *Marchantia polymorpha* reveals that R2R3MYB activation of flavonoid production in response to abiotic stress is an ancient character in land plants. *New Phytologist*, *218*, 554–566.

Allen, C. E. (1917). A chromosome difference correlated with sex differences in *Sphaerocarpos*. *Science*, *46*, 466–467.

Apostolakos, P., Galatis, B., & Mitrakos, K. (1982). Studies on the development of the air pores and air chambers of *Marchantia* paleacea I. Light-microscopy. *Annals of Botany*, *49*, 377–396.

Arun, A., Coelho, S. M., Peters, A. F., Bourdareau, S., Péres, L., et al. (2019). Convergent recruitment of TALE homeodomain life cycle regulators to direct sporophyte development in land plants and brown algae. *eLife*, *8*, e43101.

Barnes, C. R., & Land, W. J. G. (1907). Bryological papers I. the origin of air chambers—contributions from the Hull botanical laboratory, 100. *Botanical Gazette*, *44*, 197–213.

Barnes, C. R., & Land, W. J. G. (1908). Bryological papers II. The origin of the cupule of *Marchantia*—contributions from the hull botanical laboratory 120. *Botanical Gazette*, *46*, 401–409.

Benson-Evans, K. (1961). Environmental factors and bryophytes. *Nature*, *191*, 255–260.

Bergdolt, E. (1926). *Untersuchungen über Marchantiaceen*. Jena: Gustav Fischer.

Berland, H. N. W. A., Stavland, A., Jordheim, M., McGhie, T. K., et al. (2019). Auronidins are a previously unreported class of flavonoid pigments that challenges when anthocyanin biosynthesis evolved in plants. *Proceedings of the National Academy of Sciences the United States of America*, *116*, 20232–20239.

Berrie, G. K. (1963). Cytology and phylogeny of liverworts. *Evolution*, *17*, 347–357.

Bischler-Causse, H. (1989). *Marchantia L. The Asiatic and Oceanic taxa*. Berlin - Stuttgart: J. Cramer.

Bischoff, G. W. (1835). Bemerkungen über die Lebermoose vozüglich aus den gruppen der Marchantieen und Riccieen. *Nova Acta Physico Medica A C L*, *17*(2), 909–1088.
Blanchard, F. (1891). Common objects available for exhibiting the power of the microscope. *The American Monthly Microscopical Journal*, *12*, 138–139.
Bock, H. (1552). *De stirpium maxime*. Argenorati: Excudebat V. Rihelius.
Bohr, F. J. (1897). An introduction to structural botany physiologische pflanzenanatomie. *Nature*, *55*, 457–458. https://doi.org/10.1038/055457a0.
Bolleter, E. (1905). *Fegatella conica* (L) Corda. Eine morphologisch-physiologische Monographie. *Beih. Bot. Centralbl.*, *18*, 327–408.
Bowen, E. J. (1935). A note on the conduction of water in *Fibriaria bleumeana*. *Annals of Botany*, *49*, 844–848.
Bowman, J. L. (2013). Walkabout on the long branches of plant evolution. *Current Opinion in Plant Biology*, *16*, 70–77.
Bowman, J. L. (2016). A brief history of Marchantia from Greece to genomics. *Plant and Cell Physiology*, *57*, 210–229.
Bowman, J. L., Araki, T., Arteaga-Vazquez, M. A., Berger, F., Dolan, L., et al. (2016). The naming of names: Guidelines for gene nomenclature in *Marchantia*. *Plant and Cell Physiology*, *57*, 257–261.
Bowman, J. L., Briginshaw, L. N., & Florent, S. N. (2019). Evolution and co-option of developmental regulatory networks in early land plants. *Current Topics in Developmental Biology*, *131*, 35–53.
Bowman, J. L., Flores-Sandoval, E., & Kato, H. (2021). On the evolutionary origins of Land plant auxin biology. *Cold Spring Harbor Perspectives in Biology*, *a040048*.
Bowman, J. L., Kohchi, T., Yamato, K. T., Jenkins, J., Shu, S., et al. (2017). Insights into Land plant evolution garnered from the *Marchantia polymorpha* genome. *Cell*, *171*, 287–304.
Bowman, J. L., Sakakibara, K., Furumizu, C., & Dierschke, T. (2016). Evolution in the cycles of life. *Annual Review of Genetics*, *50*, 133–154.
Brodie, H. J. (1951). The splash-cup dispersal mechanism in plants. *Canadian Journal of Botany-Revue Canadienne De Botanique*, *29*, 224–234.
Burgeff, H. (1943). *Genetische Studien an Marchantia*. Jena: Gustav Fischer.
Cavers, F. (1903). Explosive discharge of antherozoids in Hepaticae. *Torreya*, *3*, 179–183.
Cavers, F. (1904). On the structure and biology of *Fegatella conica*. *Annals of Botany*, *18*, 87–120.
Clayton, W. A., Albert, N. W., Thrimawithana, A. H., McGhie, T. K., Deroles, S. C., et al. (2018). UVR8-mediated induction of flavonoid biosynthesis for UVB tolerance is conserved between the liverwort *Marchantia polymorpha* and flowering plants. *Plant Journal*, *96*, 503–517.
Courtoy, R. (1964). Recherches expérimentales sur les facteurs de la sexualisation des Thalles de *Marchantia polymorpha* L. *Bulletin de la Classes des Sciences*, *50*, 1341–1359.
de Sousa, F., Foster, P. G., Donoghue, P. C. J., Schneider, H., & Cox, C. J. (2019). Nuclear protein phylogenies support the monophyly of the three bryophyte groups (Bryophyta Schimp.). *New Phytologist*, *222*, 565–575.
Delaux, P.-M., & Schornack, S. (2021). Plant evolution driven by interactions with symbiotic and pathogenic microbes. *Science*, *371*, eaba6605.
Delwiche, C. F., & Cooper, E. D. (2015). The evolutionary origin of a terrestrial Flora. *Current Biology*, *25*, R899–R910.
Dickson, H. (1932). Polarity and the production of adventitious growing points in *Marchantia polymorpha*. *Annals of Botany*, *46*, 683–701.
Dierschke, T., Flores-Sandoval, E., Rast-Somssich, M. I., Altoff, F., Zachgo, S., & Bowman, J. L. (2021). Gamete expression of TALE class HD genes activates the diploid sporophyte program in *Marchantia polymorpha*. *eLife*, *10*, e57088.

Douin, R. (1921). Reserches sur les Marchantiées. *Revue générale de botanique*, *33*, 34–62. 99–145; 190–209.
Duckett, J. G., Ligrone, R., Renzaglia, K., & Silvia, P. (2014). Pegged and smooth rhizoids in complex thalloid liverworts (Marchantiopsida): Structure, function and evolution. *Botanical Journal of the Linnean Society*, *174*, 68–92.
Duckett, J. G., & Pressel, S. (2009). Extraordinary features of the reproductive biology of *Marchantia* at Thursley common. *Field Bryology*, *97*, 2–11.
Durand, E. J. (1908). The development of the sexual organs and Sporogonium of *Marchantia polymorpha*. *Bulletin of the Torrey Botanical Club*, *35*, 321–335.
Edwards, D., Morris, J. L., Richardson, J. B., & Kenrick, P. (2014). Cryptospores and cryptophytes reveal hidden diversity in early land floras. *New Phytologist*, *202*, 50–78.
Eklund, D. M., Ishizaki, K., Flores-Sandoval, E., Kikuchi, S., Takebayashi, Y., et al. (2015). Auxin produced by the indole-3-pyruvate pathway regulates development and gemmae dormancy in the liverwort Marchantia polymorpha. *The Plant Cell*, *27*, 1650–1669.
Eklund, D. M., Kanei, M., Flores-Sandoval, E., Ishizaki, K., Nishihama, R., et al. (2018). An evolutionarily conserved abscisic acid signaling pathway regulates dormancy in the liverwort *Marchantia polymorpha*. *Current Biology*, *28*, 3691–3699.
Fitting, H. (1939). Untersuchungen über den Einfluss von Licht und Dunkelheit auf die Entwicklung von Moosen. I. Die Brutkörper der Marchantieen. *Jahrbucher fur Wissenschaftliche Botanik*, *88*, 633–722.
Flores-Sandoval, E., Dierschke, T., Fisher, T. J., & Bowman, J. L. (2016). Efficient and inducible use of artificial MicroRNAs in *Marchantia polymorpha*. *Plant and Cell Physiology*, *57*, 281–290.
Flores-Sandoval, E., Eklund, D. M., & Bowman, J. L. (2015). A simple auxin transcriptional response system regulates multiple morphogenetic processes in the liverwort *Marchantia polymorpha*. *PLoS Genetics*, *11*, 1005207.
Flores-Sandoval, E., Eklund, D. M., Hong, S.-F., Alvarez, J. P., Fisher, T. J., et al. (2018). Class C ARFs evolved before the origin of land plants and antagonize differentiation and developmental transitions in *Marchantia polymorpha*. *New Phytologist*, *218*, 1612–1630.
Gasparrini, G. (1856). *Ricerche sulla natura dei succiatori*. Napoli: Giuseppe Dura Libraio-Editore.
Goebel, K. (1898). Uber den Offnungsmechanismus der Moos-antheridien. *Annales du Jardin botanique de Buitenzorg Supplement*, *II*, 65–72.
Goebel, K. (1902). Morphologische und biologische Bemerkungen. 11. Ueber Homologien in der Entwickelung männlicher und weiblicher Geschlechtsorgane. *Flora*, *90*, 279–305.
Goebel, K. (1930). *Organographie der Pflanzen*. Gustav Fischer, Jena: Insbesondere der Archegoniaten und Samenpflanzen.
Groenland, J. (1854). Mémoire sur la Germination de Quelques Hépatiques. *Annales de Sciences Naturelles Botanique*, *4*(1), 5–29.
Haberlandt, G. (1889). Ueber das Längenwachsthum und den Geotropismus der Rhizoiden von Marchantia und Lunularia. *Österr Bot Zeit*, *39*, 93–98.
Hansel, V. (1876). Über die Keimung der *Preissia commutata*. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe. Abt. 1. *Mineralogie, Botanik, Zoologie, Geologie und Paläontologie*, *73*, 89–97.
Haupt, G. (1932). Beiträge zur Zytologie der Gattung Marchantia I. *Zeitschrift für Induktive Abstammungs- und Vererbungslehre*, *62*, 367–428.
Hedwig, J. (1783). *Theoria Generationis et Fructificationis Plantarum Cryptogamicarum Linnaei, mere propriis Observationibus et Experimentis Superstructa*. Scientiarum, Petropoli: Academiae Imper.
Heitz, E. (1928). Das Heterochromatin der Moose I. Jahrbücher für wissenschaftliche. *Botanik*, *69*, 762–818.

Heitz, E. (1949–1951). Weitere beobachtungen an den geschelchtsmutanten, insbesondere den intersexen von *Pellia neesiana*. *Portugaliae Acta Biologica, Serie A. Morfologia, Fisiologia, Genetica e Biologia Geral*, 353–366.
Henfrey, A. (1853). XII. On the development of the spores and elaters of Marchantia polymorpha. *Transactions of the Linnean Society*, *21*, 103–110. tab. XI.
Higo, A., Kawashima, T., Borg, M., Zhao, M., López-Vidriero, I., et al. (2018). Transcription factor DUO1 generated by neofunctionalization is associated with evolution of sperm differentiation in plants. *Nature Communications*, *9*, 5283.
Hirakawa, Y., Fujimoto, T., Ishida, S., Uchida, N., Sawa, S., et al. (2020). Induction of Multichotomous branching by CLAVATA peptide in *Marchantia polymorpha*. *Current Biology*, *30*, 3833–3840.
Hirakawa, Y., Uchida, N., Yamaguchi, Y. L., Tabata, R., Ishida, S., et al. (2019). Control of haploid meristem activity by CLE peptide signaling in *Marchantia polymorpha*. *PLoS Genetics*, *15*, e1007997.
Hisanaga, T., Fujimoto, S., Cui, Y., Sato, K., Sano, R., Yamaoka, S., et al. (2021). Deep evolutionary origin of gamete-directed zygote activation by KNOX/BELL transcription factors in green plants. *eLife*, *10*, e57090.
Hisanaga, T., Okahashi, K., Yamaoka, S., Kajiwara, T., Nishihama, R., et al. (2019). A cis-acting bidirectional transcription switch controls sexual dimorphism in the liverwort. *The EMBO Journal*, *38*, e100240.
Hisanaga, T., Yamaoka, S., Kawashima, T., Higo, A., Nakajima, K., et al. (2019). Building new insights in plant gametogenesis from an evolutionary perspective. *Nature Plants*, *5*, 663–669.
Hiwatashi, T., Goh, H., Yasui, Y., Koh, L. Q., Takami, H., et al. (2019). The RopGEF KARAPPO is essential for the initiation of vegetative reproduction in *Marchantia polymorpha*. *Current Biology*, *29*, 3525–3531.
Hofmeister, W. F. B. (1862). *On the germination, development, and fructification of the higher Cryptogamia, and on the fructification of the Coniferae*. London: Ray Society.
Honkanen, S., Jones, V. A. S., Morieri, G., Champion, C., Hetherington, A. J., et al. (2016). The mechanism forming the cell surface of tip-growing rooting cells is conserved among Land plants. *Current Biology*, *26*, 3238–3244.
Honkanen, S., Thamm, A., Arteaga-Vazquez, M. A., & Dolan, L. (2018). Negative regulation of conserved RSL class I bHLH transcription factors evolved independently among land plants. *eLife*, 7, e38529.
Horst, N. A., Katz, A., Pereman, I., Decker, E. L., Ohad, N., et al. (2016). A single homeobox gene triggers phase transition, embryogenesis and asexual reproduction. *Nature Plants*, *2*, 15209.
Inoue, H. (1960). Studies in spore germination and the earlier stages of gametophyte development in the Marchantiales. *The Journal of the Hattori Botanical Laboratory*, *23*, 148–191.
Inoue, K., Nishihama, R., Araki, T., & Kohchi, T. (2019). Reproductive induction is a far-red high irradiance response that is mediated by phytochrome and PHYTOCHROME INTERACTING FACTOR in *Marchantia polymorpha*. *Plant and Cell Physiology*, *60*, 1136–1145.
Inoue, K., Nishihama, R., Kataoka, H., Hosaka, M., Manabe, R., et al. (2016). Phytochrome signaling is mediated by PHYTOCHROME INTERACTING FACTOR in the liverwort *Marchantia polymorpha*. *Plant Cell*, *28*, 1406–1421.
Ishida, S., Suzuki, H., Iwaki, A., Kawamura, S., Yamaoka, S., Kojima, M., et al. (2022). Diminished auxin signaling triggers cellular reprogramming by inducing a regeneration factor in the liverwort Marchantia polymorpha. *Plant & Cell Physiology*, *63*. https://doi.org/10.1093/pcp/pcac004.
Ishizaki, K., Chiyoda, S., Yamato, K. T., & Kohchi, T. (2008). Agrobacterium-mediated transformation of the haploid liverwort *Marchantia polymorpha* L., an emerging model for plant biology. *Plant and Cell Physiology*, *49*, 1084–1091.

Ishizaki, K., Johzuka-Hisatomi, Y., Ishida, S., Iida, S., & Kohchi, T. (2013). Homologous recombination-mediated gene targeting in the liverwort *Marchantia polymorpha* L. *Scientific Reports*, *3*, 1532.

Ishizaki, K., Mizutani, M., Shimamura, M., Masuda, A., Nishihama, R., et al. (2013). Essential role of the E3 ubiquitin ligase NOPPERABO1 in Schizogenous intercellular space formation in the liverwort *Marchantia polymorpha*. *Plant Cell*, *25*, 4075–4084.

Ishizaki, K., Nishihama, R., Ueda, M., Inoue, K., Ishida, S., et al. (2015). Development of gateway binary vector series with four different selection markers for the liverwort *Marchantia polymorpha*. *PLoS One*, *10*, e0138876.

Ishizaki, K., Nishihama, R., Yamato, K. T., & Kohchi, T. (2016). Molecular genetic tools and techniques for *Marchantia polymorpha* research. *Plant and Cell Physiology*, *57*, 262–270.

Iwasaki, M., Kajiwara, T., Yasui, Y., Yoshitake, Y., Miyazaki, M., Kawamura, S., et al. (2021). Sex determination factor and sex chromosome evolution in a haploid system. *Current Biology*, *31*, 5522–5532.

Johnson, D. S. (1904). The development and relationship of *Monoclea*. *Botanical Gazette*, *38*, 185–205.

Kamerling, Z. (1897). Zur Biologie und Physiologie der Marchantiaceen. *Flora*, *84*, 1–68.

Kanazawa, T., Morinaka, H., Ebine, K., Shimada, T. L., Ishida, S., et al. (2020). The liverwort oil body is formed by redirection of the secretory pathway. *Nature Communications*, *11*, 6152.

Kato, H., Ishizaki, K., Kouno, M., Shirakawa, M., Bowman, J. L., et al. (2015). Auxin-mediated transcriptional system with a minimal set of components is critical for morphogenesis through the life cycle in *Marchantia polymorpha*. *PLoS Genetics*, *11*, e1005365.

Kato, H., Kouno, M., Takeda, M., Suzuki, H., Ishizaki, K., et al. (2017). The roles of the sole activator-type auxin response factor in pattern formation of *Marchantia polymorpha*. *Plant and Cell Physiology*, *58*, 1642–1651.

Kato, H., Mutte, S. K., Suzuki, H., Crespo, I., Das, S., et al. (2020). Design principles of a minimal auxin response system. *Nature Plants*, *6*, 473–482.

Kelley, C. (1969). Wall projections in the sporophyte and gametophyte of *Sphaerocarpos*. *Journal of Cell Biology*, *41*, 910–914.

Kenrick, P., & Crane, P. R. (1997). *The origin and early diversification of land plants: A cladistic study*. Washington: Smithsonian Institution Press.

Kienitz-Gerloff, F. (1874). Vergleichende Untersuchungen über die Entwickelungsgeschichte des Lebermoos—Sporogoniums. *Botanische Zeitung*, *32*, 161–172. 193-204; 209-221; 225-238.

Knapp, E. (1935). Untersuchungen über die Wirkung von Röntgenstrahlen an dem Lebermoos *Sphaerocarpus*, mit Hilfe der tetraden-Analyse. I. *Zeitschrift für Induktive Abstammungs- und Vererbungslehre*, *70*, 309–349.

Kny, L. (1865). Beiträge zur Entwickelungs geschichte der laubigen Lebermoose. *Jahrbucher fur Wissenschafiliche Botanik*, *4*, 64–100.

Kohchi, T., Yamato, K. T., Ishizaki, K., Yamaoka, S., & Nishihama, R. (2021). Development and molecular genetics of *Marchantia polymorpha*. *Annual Review of Plant Biology*, *72*.

Koi, S., Hisanaga, T., Sato, K., Shimamura, M., Yamato, K. T., et al. (2016). An evolutionarily conserved plant RKD factor controls germ cell differentiation. *Current Biology*, *26*, 1775–1781.

Kreh, W. (1909). Über die Regeneration der Lebermoose. Nova Acta Abh. der Kaiserl. Leop.-Carol. *Deutschen Akademie der Naturforscher*, *90*, 216–301.

Kubo, H., Nozawa, S., Hiwatashi, T., Kondou, Y., Nakabayashi, R., et al. (2018). Biosynthesis of riccionidins and marchantins is regulated by R2R3- MYB transcription factors in *Marchantia polymorpha*. *Journal of Plant Research*, *131*, 849–864.

Kubota, A., Ishizaki, K., Hosaka, M., & Kohchi, T. (2013). Efficient *Agrobacterium*-mediated transformation of the liverwort *Marchantia polymorpha* using regenerating thalli. *Bioscience Biotechnology and Biochemistry*, *77*, 167–172.
Kubota, A., Kita, S., Ishizaki, K., Nishihama, R., Yamato, K. T., et al. (2014). Co-option of a photoperiodic growth-phase transition system during land plant evolution. *Nature Communications*, *5*, 3668.
Lagerkrantz, U., Billhardt, A., Rousku, S. N., Ljung, K., & Eklund, D. M. (2020). Nyctinastic thallus movement in the liverwort *Marchantia polymorpha* is regulated by a circadian clock. *Scientific Reports*, *10*, 8658.
LaRue, C. D., & Narayanaswami, S. (1957). Auxin inhibition in the liverwort *Lunularia*. *New Phytologist*, *56*, 61–70.
Lee, J.-H., Lin, H., Joo, S., & Goodenough, U. (2008). Early sexual origins of Homeoprotein Heterodimerization and evolution of the plant KNOX/BELL family. *Cell*, *133*, 829–840.
Leitgeb, H. (1874). *Untersuchungen über die Lebermoose. Heft I. Blasia pusilla*. Jena: O. Deistung's Buchhandlung.
Leitgeb, H. (1877a). Die Keimung der Lebermoossporen in ihrer Beziehung zum Lichte. *Sitzb Akad Wiss Wien*, *74*, 425–436. Tafel.
Leitgeb, H. (1877b). *Untersuchungen ueber die Lebermoose III. Heft. Dir Frondosen Jungermannieen*. Jena: O. Deistung's Buchhandlung (Hermann Dabis).
Leitgeb, H. (1879). *Untersuchungen über die Lebermoose. Heft IV. Die Riccieen*. Graz: Leuschner & Lubensky.
Leitgeb, H. (1880a). Die Athemöffnungen der Marchantiaceen Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. *Wien*, *81*, 40–54.
Leitgeb, H. (1880b). Die Inflorescenzen der Marchantiaceen Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. *Wien*, *81*, 123–143.
Leitgeb, H. (1881). *Untersuchungen über die Lebermoose Heft VI. Die Marchantieen*. Jena: Leuschner & Lubensky.
Lidforss, B. (1904-5). Über die Reizbewegungen der Marchantia-Spermatozoiden. *Jahrbucher fur Wissenschaftliche Botanik*, *41*, 65–87.
Ligrone, R., Duckett, J. G., & Renzaglia, K. S. (1993). The gametophyte-sporophyte junction in land plants. *Advances in Botanical Research*, *19*, 231–317.
Linde, A.-M., Eklund, D. M., Kubota, A., Pederson, E. R. A., Holm, K., et al. (2017). Early evolution of the land plant circadian clock. *New Phytologist*, *216*, 576–590.
Lorbeer, G. (1936). Die Umwandlung eines haploiden, genotypisch weiblichen Gametophyten von *Sphaerocarpus Donnellii* in einen männlichen mit Hilfe von Röntgenstrahlen. *Planta*, *25*, 70–83.
Maravolo, N. C. (1976). Polarity and localization of auxin movement in hepatic, *Marchantia polymorpha*. *American Journal of Botany*, *63*, 526–531.
Marchant, J. (1713). *Nouvelle Découverte des Fleurs et des Graines D'une Plante rangée par les Botanistes fous le genre du Lichen* (pp. 229–234). Année: Histoire de l'Académie royale des sciences, avec les mémoires de mathématique et de physique, pour la meme cette Académie. MDCCXIII.
McConaha, M. (1941). Ventral structures effecting capillarity in the Marchantiales. *American Journal of Botany*, *28*, 301–306.
Menge, F. (1930). Die Entwicklung der Keimpflanzen von *Marchantia polymorpha* L. und Plasgiochasma rupestre. *Flora*, *124*, 423–478.
Mirbel, C.-F. (1835). Researches anatomiques et physiologiques sur le *Marchantia polymorpha*, pour servir a l'histoire du tissu cellulaire, de l'épiderme et des stomates. *Mémoires de l'Académie Royale Societie de l'Institut de France*, *13*, 337–436.
Molisch, H. (1884). Untersuchungen über den Hydrotropismus. *Sitzb Akad Wiss Wien*, *88*, 897–942.
Molisch, H. (1922). *Pflanzenphysiologie als Theorie der Gärtnerei*. Jena: Gustav Fischer.

Montgomery, S. A., Tanizawa, Y., Galik, B., Wang, N., Ito, T., et al. (2020). Chromatin organization in early land plants reveals an ancestral association between H3K27me3, transposons, and constitutive heterochromatin. *Current Biology*, *30*, 573–588.
Morris, J. L., Puttick, M. N., Clark, J. W., Edwards, D., Kenrick, P., et al. (2018). The timescale of early land plant evolution. *Proceeding of the National Academy of Sciences USA*, *115*, E2274–E2283.
Mottier, D. M. (1891). Notes on the apical growth of liverworts. *Botanical Gazette*, *16*, 141–143.
Muggoch, H., & Walton, J. (1942). On the dehiscence of the antheridium and the part played by surface tension in the dispersal of spermatocytes in Bryophyta. *Proceedings of the Royal Society of London. Series B*, *130*, 448–461. plate 414.
Nagai, I. (1915). Ueber roten Pigmentbildung bei einigen *Marchantia*-Arten. *Botanical Magazine Tokyo*, *29*, 90–98.
Nagai, I. (1919). Induced adventitious growth in the Gemmae of *Marchantia*. *The Botanical Magazine*, *33*, 99–109.
Nägeli, K. (1842). Botanische Beiträge. *Linnea*, *16*, 237–285.
Nägeli, K. (1845). Wachsthumgeschichte der Laub- und Lebermoose. *Zeitschrift fur Wissenschaftliche Botanik*, *2*, 138–210.
Naramoto, S., Jones, V. A. S., Trozzi, N., Sato, M., Toyooka, K., et al. (2019). A conserved regulatory mechanism mediates the convergent evolution of plant shoot lateral organs. *PLoS Biology*, *17*, 3000560.
Nishihama, R., Ishida, S., Urawa, H., Kamei, Y., & Kohchi, T. (2016). Conditional gene expression/deletion systems for *Marchantia polymorpha* using its own heat-shock promoter and Cre/loxP-mediated site-specific recombination. *Plant and Cell Physiology*, *57*, 271–280.
Nishihama, R., Ishizaki, K., Hosaka, M., Matsuda, Y., Kubota, A., et al. (2015). Phytochrome-mediated regulation of cell division and growth during regeneration and sporeling development in the liverwort *Marchantia polymorpha*. *Journal of Plant Research*, *128*, 407–421.
Nishiyama, T., Wolf, P. G., Kugita, M., Sinclair, R. B., Sugita, M., et al. (2004). Chloroplast phylogeny indicates that bryophytes are monophyletic. *Molecular Biology and Evolution*, *21*, 1813–1819.
Oda, K., Yamato, K., Ohta, E., Nakamura, Y., Takemura, M., et al. (1992). Gene Organization deduced from the complete sequence of liverwort *Marchantia polymorpha* mitochondrial-DNA - a primitive form of plant mitochondrial genome. *Journal of Molecular Biology*, *223*, 1–7.
O'Hanlon, M. E. (1926). Germination of spores and early stages in development of gametophyte of *Marchantia polymorpha*. *Botanical Gazette*, *82*, 215–222.
Ohno, S. (1970). *Evolution by gene duplication*. New York: Springer-Verlag.
Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H., Sano, T., et al. (1986). Chloroplast Gene Organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. *Nature*, *322*, 572–574.
Oppenheimer, H. (1922). Das Unterbleiben der Keimung in den Behältern der Mutterpflanze. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften*, *131*, 279–312.
Peirce, G. J. (1902). Forcible discharge of the Antherozoids in *Asterella californica*. *Bulletin of the Torrey Botanical Club*, *29*, 374–382.
Pettersson, B. (1940). Experimentelle untersuchungen über die euanemochore verbreitung der sporenpflanzen. *Acta Botanica Fennica*, *25*, 1–102.
Pfeffer, W. (1871). Studien über Symmetric und spezifische Wachstumsursachen. *Arbeiten Des Botanischen Instituts in Würzburg*, *1*, 77–98.
Pressel, S., & Duckett, J. G. (2019). Do motile spermatozoids limit the effectiveness of sexual reproduction in bryophytes? Not in the liverwort *Marchantia polymorpha*. *Journal of Systematics and Evolution*, *57*, 371–381.

Proust, H., Honkanen, S., Jones, V. A. S., Morieri, G., Prescott, H., et al. (2016). RSL class I genes controlled the development of epidermal structures in the common ancestor of land plants. *Current Biology*, *26*, 93–99.

Puttick, M. N., Morris, J. L., Williams, T. A., Cox, C. J., Edwards, D., et al. (2018). The interrelationships of Land plants and the nature of the ancestral Embryophyte. *Current Biology*, *28*, 733–745.

Qiu, Y. L., Li, L. B., Wang, B., Chen, Z. D., Knoop, V., et al. (2006). The deepest divergences in land plants inferred from phylogenomic evidence. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 15511–15516.

Radhakrishnan, G. V., Keller, J., Rich, M. K., Vernié, T., Mbadinga, D. L. M., et al. (2020). An ancestral signalling pathway is conserved in intracellular symbioses-forming plant lineages. *Nature Plants*, *6*, 280–289.

Rimington, W. R., Duckett, J. G., Field, K. J., Bidartondo, M. I., & Pressel, S. (2020). The distribution and evolution of fungal symbioses in ancient lineages of land plants. *Mycorrhiza*, *30*, 23–39.

Robinson, W. (1910). *Alpine flowers for gardens—Rock, wall, marsh plants, and mountain shrubs*. London: John Murray.

Romani, F., Baniç, E., Florent, S. N., Kanazawa, T., Goodger, J. Q. D., et al. (2020). Oil body formation in *Marchantia polymorpha* is controlled by MpC1HDZ and serves as a defense against arthropod herbivores. *Current Biology*, *30*, 2815–2828.

Romani, F., & Moreno, J. E. (2021). Molecular mechanisms involved in functional macroevolution of plant transcription factors. *New Phytologist*, *230*, 1345–1353.

Rövekamp, M., Bowman, J. L., & Grossniklaus, U. (2016). *Marchantia* MpRKD regulates the gametophyte- sporophyte transition by keeping egg cells quiescent in the absence of fertilization. *Current Biology*, *26*, 1782–1789.

Ruge, G. (1893). Beiträge zur Kenntniss der vegetationsorgane der lebermoose. *Flora*, *77*, 279–312.

Sachs, J. V. (1882). Ueber orthotrope und plagiotrope pflanzentheile. *Arbeiten des botan. Institut Würzburg*, *2*, 1004–1064.

Sakakibara, K., Ando, S., Yip, H. K., Tamada, Y., Hiwatashi, Y., et al. (2013). KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants. *Science*, *339*, 1067–1070.

Sakakibara, K., Nishiyama, T., Deguchi, H., & Hasebe, M. (2008). Class 1 KNOX genes are not involved in shoot development in the moss *Physcomitrella patens* but do function in sporophyte development. *Evolution & Development*, *10*, 555–566.

Sauret-Güeto, S., Frangedakis, E., Silvestri, L., Rebmann, M., Tomaselli, M., et al. (2020). Systematic tools for reprogramming plant gene expression in a simple model, *Marchantia polymorpha*. *ACS Synthetic Biology*, *9*, 864–882.

Schmidel, C. C. (1762). *Icones Plantarum et analyses partium aeri incisae atque vivis coloribus insgnitae adiectis indicibus nominum necessariis figurarum explicationibus et brevibus animaduersionibus*. Nürnberh: Johann Christoph Keller.

Schönherr, J., & Ziegler, H. (1975). Hydrophobic Cuticular ledges prevent water entering the air pores of liverwort thalli. *Planta*, *124*, 51–60.

Schostakowitsch, W. (1894). Über die reproduction und regenerationserscheinungen bei den Lebermoosen. *Flora*, *79*, 350–384.

Schuster, R. M. (1992). *The Hepaticae and Anthocerotae of North America. Vol. VI*. New York: Columbia University Press.

Schwann, T., & Schleiden, M. J. (1847). *Microscopical researches into the accordance in the structure and growth of plants and animals*. London: Sydenham Society.

Shimamura, M. (2016). *Marchantia polymorpha*: Taxonomy, phylogeny and morphology of a model system. *Plant and Cell Physiology*, *57*, 230–256.

Skutch, A. F. (1929). Early stages of plant succession following forest fires. *Ecology*, *10*, 177–190.
Smee, A. (1872). *My garden, its plan and culture together with a general description of its geology, botany, and natural history*. London: Bell and Daldy.
Stahl, E. (1882). Ueber der Einfluss des sonnigen oder schattigen Standortes auf die Ausbildung der Laubbätter. *Jenaische Zeitschrift für Naturwissenschaft*, *16*, 162–200.
Stahl, E. (1888). Pflanzen und Schnecken: Biologische Studie über die Schutzmittel der Pflanzen gegen Schneckenfrass. *Jenaische Zeitschrift für Naturwissenschaft*, *22*, 557–684.
Strasburger, E. (1869). Die Geschlechtsorgane und die Befruchtung bei *Marchantia polymorpha* L. *Jahrbucher fur Wissenschaftliche Botanik*, 7, 409–422.
Strother, P. K., & Taylor, W. A. (2018). The evolutionary origin of the plant spore in relation to the antithetic origin of the plant sporophyte. In M. Krings, C. J. Harper, N. R. Cúneo, & G. W. Rothwell (Eds.), *Transformative paleobotany: Papers to commemorate the life and legacy of Thomas N. Taylor* (pp. 3–20). Amsterdam, Netherlands: Elsevier.
Strullu-Derrien, C., Selosse, M.-A., Kenrick, P., & Martin, F. M. (2018). The origin and evolution of mycorrhizal symbioses—From palaeomycology to phylogenomics. *New Phytologist*, *220*, 1012–1030.
Sugano, S. S., Nishihama, R., Shirakawa, M., Takagi, J., Matsuda, Y., et al. (2018). Efficient CRISPR/Cas9-based genome editing and its application to conditional genetic analysis in *Marchantia polymorpha*. *PLoS One*, *13*, e0205117.
Sugano, S. S., Shirakawa, M., Takagi, J., Matsuda, Y., Shimada, T., et al. (2014). CRISPR/Cas9-mediated targeted mutagenesis in the liverwort *Marchantia polymorpha* L. *Plant and Cell Physiology*, *55*, 475–481.
Suire, C., Bouvier, F., Backhaus, R. A., Begu, D., Bonneu, M., et al. (2000). Cellular localization of isoprenoid biosynthetic enzymes in *Marchantia polymorpha*. Uncovering a new role of oil bodies. *Plant Physiology*, *124*, 971–978.
Suzuki, H., Harrison, C. J., Shimamura, M., Kohchi, T., & Nishihama, R. (2020). Positional cues regulate dorsal organ formation in the liverwort *Marchantia polymorpha*. *Journal of Plant Research*, *133*, 311–321.
Suzuki, H., Kohchi, T., & Nishihama, R. (2021). Auxin biology in Bryophyta: A simple platform with versatile functions. *Cold Spring Harbor Perspectives in Biology*, a040055.
Takizawa, R., Hatada, M., Moriwaki, Y., Abe, S., Yamashita, Y., et al. (2021). Fungal-type terpene synthases in *Marchantia polymorpha* are involved in sesquiterpene biosynthesis in oil body cells. *Plant & Cell Physiology*. https://doi.org/10.1093/pcp/pcaa175.
Tanaka, M., Esaki, T., Kenmoku, H., Koeduka, T., Kiyoyama, Y., et al. (2016). Direct evidence of specific localization of sesquiterpenes and marchantin a in oil body cells of *Marchantia polymorpha* L. *Phytochemistry*, *130*, 77–84.
Tarén, N. (1958). Regulating the initial development of Gemmae in *Marchantia polymorpha*. *The Bryologist*, *61*, 191–204.
Taylor, T. (1837). De Marchantieis. *Transactions of the Linnean Society*, *17*, 375–395.
Téodoresco, E. C. (1899). Influence de l'acide carbonique sur la forme et la structure des plantes. *Revue Générale de Botanique*, *11*, 445–470.
Téodoresco, E. C. (1929). Observations sur la croissance des plantes aux lumiéres de diverses longeurs d'ondé. *Annales des sciences naturelles Botanique*, *10*(11), 201–335.
Thamm, A., Saunders, T. S., & Dolan, L. (2020). MpFEW RHIZOIDS1 miRNA-mediated lateral inhibition controls rhizoid cell patterning in *Marchantia polymorpha*. *Current Biology*, *30*, 1905–1915.
Thomas, R. J. (1980). Cell elongation in hepatics: The seta system. *Bulletin of the Torrey Botanical Club*, *107*, 339–345.
Thuret, G. (1851). Recherches sur les Zoospores des Algues et les Anthéridies des Cryptogames. *Annales de Sciences Naturelles Botanique*, *3.16*, 5–39, Planches 31-15.

Torrey, R. H. (1932). *Marchantia polymorpha* after forest fires. *Torreya*, *32*(9–10), 128–129.
Tsuboyama, S., Nonaka, S., Exura, H., & Kodama, Y. (2018). Improved G-AgarTrap—A highly efficient transformation method for intact gemmalings of the liverwort *Marchantia polymorpha*. *Scientific Reports*, *8*, 10800.
Tsuzuki, M., Futagami, K., Shimamura, M., Inoue, C., Kunimoto, K., et al. (2019). An early arising role of the MicroRNA156-529-SPL module in reproductive development revealed by the liverwort *Marchantia polymorpha*. *Current Biology*, *29*, 3307–3314.
Unger, F. (1837). Weitere Beobachtungen über die Samenthiere der Pflanzen. *Nova Acta physico medica A C L*, *18*, 786–796.
Vöchting, H. (1885). Über die regeneration der Marchantieen. *Jahrbucher fur Wissenschaftliche Botanik*, *16*, 367–414.
Wann, F. B. (1925). Some of the factors involved in the sexual reproduction of *Marchantia polymorpha*. *American Journal of Botany*, *12*, 307–318.
Wickett, N. J., Mirarab, S., Nguyen, N., Warnow, T., Carpenter, E., et al. (2014). Phylotranscriptomic analysis of the origin and early diversification of land plants. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, E4859–E4868.
Xu, B., Taylor, L., Pucker, B., Feng, T., Glover, B. J., et al. (2021). The land plant-specific MIXTA-MYB lineage is implicated in the early evolution of the plant cuticle and the colonization of land. *New Phytologist*, *229*, 2324–2338.
Yamaoka, S., Nishihama, R., Yoshitake, Y., Ishida, S., Inoue, K., et al. (2018). Generative cell specification requires transcription factors evolutionarily conserved in land plants. *Current Biology*, *28*, 479–486.
Yamaoka, S., Takenaka, M., Hanajiri, T., Shimizu-Ueda, Y., Nishida, H., et al. (2004). A mutant with constitutive sexual organ development in *Marchantia polymorpha* L. *Sexual Plant Reproduction*, *16*, 253–257.
Yamato, K. T., Ishizaki, K., Fujisawa, M., Okada, S., Nakayama, S., et al. (2007). Gene organization of the liverwort Y chromosome reveals distinct sex chromosome evolution in a haploid system. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 6472–6477.
Yasui, Y., Tsukamoto, S., Sugaya, T., Nishihama, R., Wang, Q.-X., et al. (2019). GEMMA CUP-ASSOCIATED MYB1, an Ortholog of axillary meristem regulators, is essential in vegetative reproduction in *Marchantia polymorpha*. *Current Biology*, *29*, 3987–3995.
Young, S. B., & Kläy, J.-R. (1971). Bryophytes in the 1969 crater of Deception Island, Antarctica: An apparent case of rapid long-distance dispersal. *Ohio Journal of Science*, *71*, 358–362.
Leclerc du Sablon M., (1885). Recherches sur le développement du sporogone des hépatiques. Bulletin de la Société Botanique de France, 7, t. 2: 126–180.