

A Brief History of *Marchantia* from Greece to Genomics

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While written accounts of plants date back thousands of years, due to the degradation of scientific literature during the dark ages descriptions descended from Greek writings are sometimes equivocal as to species identity. Such is the case with *Marchantia* in the pre-Renaissance literature; however, indisputable illustrations of *Marchantia polymorpha* were made as early as the mid-15th century, beginning a rich historical literature on its taxonomy, development and physiology. In this review, I present three vignettes, each of which are themselves abbreviated due to space constraints. The first presents the role of *Marchantia* and related liverwort species in the discovery of sex in cryptogams, from the elucidation of liverwort life cycles the 18th century to the sequence of the Y chromosome in the 21st. A second vignette describes the use of *M. polymorpha* as a model organism in the early 19th century debate concerning the cellular nature of organisms and the origin of new cells—an endeavor that provided us with Charles-François Brisseau de Mirbel's *mémoire* containing beautiful, if slightly fanciful, illustrations of the *Marchantia* life cycle. The final vignette chronicles the use of *M. polymorpha* gemmae over the past two centuries to elucidate the mechanism by which a dorsiventral body plan is established from an initially apolar gemma. While only covering a fraction of the literature available, these vignettes provide a glimpse of historical and recent discoveries available upon which to build a molecular genetic and genomic understanding of *Marchantia*.

Keywords: Cell theory • History • *Marchantiab* • Polarity • Sex determination.

Early Descriptions of Liverworts

While man's initial knowledge of plants pre-dates the dawn of our species, the seeds of scientific investigation began in the 6th to 4th centuries BC in Greece with Ionian philosophers who based their ideas on observation and attempted to explain the world as a rational result of natural forces, a significant departure from invoking the supernatural (Morton 1981). In the botanical realm this included lists of plants and their uses. One of the earliest known lists was assembled by Diokles of Karystos in about 350 BC and is thought to have consisted of a compendium of plants followed by a description of their habitats and medicinal uses (Singer 1927). While all original accounts of these early writings have been lost, later authors, from the

ancient Greeks through the Renaissance, continued this format referred to as a Herbal (Arber 1912). The historical representations of liverworts begin with nebulous descriptions and illustrations from antiquity through the dark ages, only becoming realistic enough to be assigned unambiguously to specific species in the Renaissance. While the majority of this review is focused on the genus *Marchantia*, other liverwort taxa are mentioned and their phylogenetic relationships with *Marchantia* are depicted in Fig. 1.

Greek and Roman antiquity

Theophrastus, a native of the island of Lesbos, is considered the 'father of botany', but it is probable at least some of Theophrastus' writings were derived from or influenced by those of Diokles (Morton 1981). His botanical works—the *Historia Plantarum* (*Enquiry into Plants*) and *Causae Plantarum* (*Causes of Plants*)—contain both notes on plant growth and physiology and descriptions of many plant species, native and foreign, with many of the latter being brought back to Greece during the conquests of Alexander the Great (356–323 BC) (Scarborough 1978, Morton 1981). This influx of foreign plants, animals and ideas undoubtedly broadened the scope and thinking of biology in Greece at this time.

Lichen (λειχήν) is a word of Greek origin used by Theophrastus in *De Causis Plantarum* (Book V 9.10) to signify a superficial growth on the bark of olive trees (Smith 1921). It is suggested that the term lichen comes from the Greek 'to lick', as the plants 'lick' the bark and stones. While Theophrastus' original use probably referred to an organism that today we would recognize as a lichen, it is assumed by Lindberg (Lindberg 1877) that the term λειχήν was more broadly applied to many plants with a thalloid body plan, including liverworts. Thus, in early texts and floras based on the work of Theophrastus and other Greek and Roman authors who followed, liverworts and hornworts are referred to as Lichens.

While Theophrastus' work was not a herbal, it provided the basis for later works, such as that of Krateus, the medical attendant of Mithridates, who produced both a written Herbal and a second novel one in which plants were depicted in figures rather than described in words, making Krateus the father of plant illustration (Pliny and Holland 1634). Krateus' illustrated format of herbal was followed for the next 1,500 years with the most influential herbal being that of Pedanius Dioscorides of Anazarba, a Greek physician and botanist who wrote a five volume encyclopedia on herbal medicine, *Peri Hules Iatrikes*,

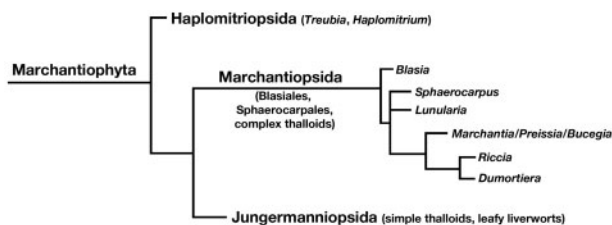


Fig. 1 Relationships of the various liverwort (Marchantiophyta) taxa mentioned in the text. Adapted from phylogenies reconstructed using molecular data (Forrest et al. 2006, He-Nygrén et al. 2006).

or *De Materia Medica* in its Latin translation (Stannard 1965, Stannard 1999). Undoubtedly Dioscorides based his work on those of his predecessors, including Theophrastus, Krateus and Nicander of Colophon, and it remains the primary historical source of medicines used by the ancient Greeks and through *De Materia Medica* and its successors, botany and medicine would be closely entwined until the beginning of the 19th century. *De Materia Medica* was not originally illustrated, and was copied through the centuries, first in Greek then translated into Latin and Arabic during the dark ages. During this time, illustrations were added and changes accumulated through the centuries via errors introduced by transcribers who had little, if any, knowledge of the plants described. The oldest surviving version of *De Materia Medica* is contained within the *Juliana Anicia Codex* (also known as the *Codex Vindobonensis* or the *Vienna Dioscorides*), which was completed in Constantinople around 512 (Dioscorides 512). While the illustrations of the *Juliana Anicia Codex* vary in quality, the best are naturalistic, probably copied from earlier Greek texts that no longer exist, since by this time in the dark ages naturalistic drawing was a lost art. Lichen is described and illustrated in Chapter 53 of Book IV (fol. 216v.), with the following text from John Goodyer's translation of *De Materia Medica* into English, a task he completed in 1655, but which was not published until the 20th century (Dioscorides et al. 1933):

4.53 Leichen, Lichen

'Lichen, that which grows upon rocks, but some call it Bryon, is a moss sticking to moist rocks. This being laid on doth stop ye fluxes of blood, & it doth help the Ictericall [jaundice] being laid on with honey, and doth help also the rhumes of ye mouth, & ye tongue.'

Many subsequent authors assumed that the description was of a liverwort, specifically *Marchantia* as it is the most common liverwort in human disturbed habitats in Europe. While the text of Dioscorides could be describing a liverwort, the accompanying illustration, where a nondescript patch of green plants is growing on top of a pile of stones, could be depicting either a true lichen or a liverwort, but more closely resembling the former. While the texts of different translations of *De Materia Medica* did not change much for the next millenium, the words being slavishly copied over the centuries, the illustrations, initially based on drawing from nature, degraded into unrecognizable sketches impossible to reconcile with actual plants they were supposed to represent (Sachs 1890, Morton 1981).

While Dioscorides' description is ambiguous, that of Pliny is more readily interpreted as a marchantialean liverwort. Pliny the Elder (23–79 AD) was a Roman naturalist, author and military commander in the early Roman Empire. His *Naturalis Historia* is a 37 volume encyclopedia of the knowledge of his time, completed in 77 AD, 2 years before his death in an ill-fated attempt to rescue friends in Herculaneum from the eruption of Mt Vesuvius. While Pliny and Dioscorides wrote contemporaneously they were unlikely to have been aware of each other's works. The relevant passage in *Naturalis Historia* is in Book XXVI, where Lichen is described in Chapter IIII from Philemon Holland's English translation (Pliny and Holland 1634):

'... but the hearbe Liverwort excellent all the rest, which therupon tooke the name Lichen: it groweth upon stonie grounds, with broad leaves beneath about the root, having one stalke and the same small, at which there hang downe long leaves; and surely this a proper hearbe also to wipe away all markes and cicatrices in the skin, it is be bruised and laid upon them with honey: Another kind of Lichen or Liverwort there is, cleaving wholly fast upon rocks and stones in a manner of mosse, which also is singular for those tettars, beeing reduced into a liniment. This hearbe likewise stauncheth the flux of bloud in greene wounds, if the juice be dropped into them: and in a liniment, it serveth well to be applied unto apostumat places: the jaundise it healeth, in case of the mouth and tongue be rubbed and annointed with it and hony together: but in this cure the patients must have in charge, To bath in salt water, to annoint themselves with the oile of almonds, and in any case to abstaine from all salads and pothearbs of the garden.'

Based on his use of the term, it can be assumed that Holland accepted that the plant described by Dioscorides and Pliny was a liverwort.

The Renaissance

As stated by Sachs (Sachs 1890) '... the botanical literature of the middle ages grows less and less valuable ... as they are deficient in ideas ... [and] had sunk so low, that not only were the figures embellished with fabulous additions ... sometimes drawn purely from fancy, but the meagre descriptions of quite common plants were not taken from nature, but borrowed from earlier authorities and eked out with superstitious fictions.' At the advent of the Renaissance this began to change, first in northern Italy and subsequently spreading to the remainder of Europe (Sprague and Nelves 1931, Morton 1981). The unprecedented wealth of merchants and bankers from the commercial cities of Venice, Florence, Genoa and Milan led to considerable political power and independence, which was used to support cultural change and scientific inquiry. The wealth was in part derived from these city states controlling trade routes, which also brought knowledge of an unparalleled number of new plant species. At Padua, and then elsewhere, the establishment of positions within Italian universities to study botany explicitly, and the universities' independence from the influence of the Church, allowed botanical studies to flourish. By 1546 botanical gardens were established in Pisa, Padua and Florence, a trend that spread across Italy and then more broadly in Europe. It is not surprising that the early botanical Renaissance occurred around Padua given the relationship of

botany to medicine and the blossoming of medical schools at this time in northern Italy.

The earliest known illustration that is undoubtedly *Marchantia polymorpha* can be found in a copy of *Libre de Simplicibus*, known as *Marciana Codex lat. VI 59*, which was compiled by Italian physician Benedetto Rinio in 1419, and illustrated by an otherwise unknown artist, Andrea Amadio (Blunt and Raphael 1994, Pächt 1950). In *Marciana Codex lat. VI 59*, naturalistic illustrations of plants are presented along with their names in Greek, Arabic, Latin and other languages. While the text was compiled in 1419, the illustrations may not have been completed until the mid-15th century, as the manuscript is dated 1445–1448.

The advent of the printing press facilitated a demand for widely available books, and in the first half of the 16th century after the Renaissance had spread northward, three German botanists, Otto Brunfels, Leonhart Fuchs and Jerome Hieronymus Bock, produced Herbals. In these they provided both figures and descriptions often based on their own observations of local plants, although they still primarily focused on plants ‘useful’ to man. In Fuchs’ *De Historia Stirpium*, first published in 1542, is the first conclusive depiction of *M. polymorpha* (female) in printed literature [Fig. 2; (Fuchs et al. 1542)]. Fuchs had previously chastised earlier authors for changing the name from Lichen to Hepatica when he can find no evidence in the writings of the Greeks of Lichen as a treatment for liver ailments, but rather all the Greek descriptions are of using extracts of Lichen as a topical ointment.

‘It is already sufficiently evident that the Lichen of the ancients differs in no respect from the Hepatica of the moderns. . . . Lichen has been wrongly called Hepatica by the younger writers, inasmuch as, according to the testimony of the ancients, it is not serviceable to the liver. . . . learn from the teachings of Dioscorides and Pliny that this herb Lichen . . . is not to be taken within, but rather applied externally . . .’

Fuchs in (Brunfels 1531), transl. Howe (Howe 1894)

The moniker ‘liverwort’ has its origins in the ‘doctrine of signatures’, which states that plants that resemble a particular part of the body can be used to treat ailments of that part of the body. Since thalloid liverworts vaguely resembled the human liver, the term Hepatica was borrowed from the Greek and Latin word for liver [Hepaticus], and this was extended into other languages, e.g. Jecoraria (also Latin), liverwort (English), leberkraut (German), hepatique (French), fegetella (Italian), Azez alsacher (Arabic), etc. Unfortunately, these names, derived from a fanciful superstition of the dark ages, are now indelibly associated with liverworts.

By the mid-16th century the number of maladies that liverworts were purportedly effective against grew well beyond those mentioned by Dioscorides, as exemplified by this entry in Rembert Dodoens’ *Cruydeboeck* (Dodoens et al. 1578):

‘The decoction of liverworte, swageth the inflammation of the liver, openeth the stoppings of the same and is very good against fever tertians, and all inflammations of blood. This herbe (as Dioscorides and Plinie writeth) used when it is yet greene, and layd upon wounds, stoppeth the superfluous bleeding of the same

and preserueth them both from inflammation and apostemation. The same also heale all foule scurffes and spreading scabbes as the pockes, and wilde-fire, and taketh away the marques and scarres made with hoate irons, if it be pounce with hony and layde thereupon. The same boyled in wine, and holden in the mouth, stoppeth the Catarrhes, that is, a distilling or falling downe of Reume, or water and flegme from the brayne to the throte.’

Very different purported uses for *Marchantia* were conceived in the Americas, with reports of use of *Marchantia* (paillahue in the Mapundungun language) in the preparation of a love potion in pre-Columbian Chile, as a ‘remedy to soften the will of the desired person’ (Mösbach 1992). It is likely the Mapundungun are referring to one of the native *Marchantia* species of southern Chile, e.g. *M. foliacea* or *M. berteroaana*. Likewise, *Marchantia* was also reported as an aphrodisiac by the Iroquois of the present-day northeastern USA and eastern Canada (Herrick 1995), suggesting it may have been a common belief throughout the pre-Columbian Americas.

Marchantia Acquires its Name

Jean Marchant (1650–1738) was the son of Nicolas Marchant, the sole botanist among the founding members of the Académie Royale des Sciences (Paris). Under the auspices of the Académie, the elder Marchant undertook the preparation of a *Historie des Plantes* in 1667, a project in which the younger Marchant joined and continued after his father’s death in 1678. Unfortunately, the project was abandoned by the Académie in 1694, and while Jean Marchant continued to prepare botanical descriptions on his own, the work was never published. However, some of the descriptions did appear in the Académie’s *Mémoires*, one of which is the 1713 description of ‘*Marchantia stellata*’, in which he names the genus *Marchantia* in memory of his father (Marchant 1713).

‘We will establish for this plant a new genus that we will call Marchantia, named from the late Mr. Marchant, my father, who had the honor of being the first botanist occupying a place in the Academy, when the king created the company in 1666. We advise those who want to have the pleasure of seeing the flower [archegoniophore] of Marchantia stellata to look for it after stormy weather or warm rain.’

Marchant (1713) p. 233; transl. J. L. Bowman and Pauline Jullien

While *Marchantia* is often pronounced with a hard ‘k’ (even in France), it has been argued that it should be pronounced with a soft ‘ch’ in accordance with its derivation from the French name ‘Marchant’ (Motte 1949).

In Marchant’s description, he states that he is only describing *Lichen petraeus stellatus*, which we now know as the female *M. polymorpha* (Fig. 3). He mentions that others have described similar plants as being the male of the same species (Bauhin et al. 1651), but he will not enter into the arguments as to whether they represent different species or varieties of the same species. This debate continued until archegonia and antheridia were interpreted as female and male organs, respectively (Schmidel 1762, Hedwig 1783). Gemmae gups are clearly present on the thalli in Marchant’s illustration, but



Fig. 2 Fuchs (p. 476) describes *Marchantia* as bearing ‘stellate capitula’ when he collected it in July (Fuchs et al. 1542). Unusual for the time, Fuchs gave credit to the artists, depicting them in portraits at the end of the work: Albrecht Meyer who drew the plants under Fuchs’ guidelines of (continued)

dichotomous branching is not clearly depicted, and thus the gemmae cups appear randomly distributed. However, several notable observations were made by Marchant, including the most detailed description to date of the female reproductive tissues, e.g. that there are generally nine rays on the archegoniophore, and Marchant was the first to describe elaters and postulate their function.

'At the same time that the flower [he considered the archegoniophore a flower] blooms, you will discover inside a tuft of very fine silky threads (e) of yellow-gold color . . . which gradually lengthening & visibly flourishing leak an infinity of very small yellow nearly round particles (f). . . however, we can see very clearly in our plant flower, the silken nets grow & stretch like would do a group of small threads exposed to the sun's heat, and that the seeds [he considered the spores as seeds] of the same plant spread continuously as the atoms in the air, which makes the mechanical marvelescence of this flower.'

Marchant (1713) p. 231–233; transl. J. L. Bowman and Pauline Jullien

Sex in the Cryptogams

During the Enlightenment, a cultural movement among intellectuals in the 17th–18th centuries that included the Scientific Revolution, many older ideas of the world were overturned, including the place of the earth within the solar system and the place of man in biology. The discovery of sex in plants provided a fresh perspective of plant biology and spawned intense investigation to uncover mechanisms. The lack of obvious homologies or analogies between the reproductive organs across all plants led to a classification, which lasted until well into the 20th century, based on the presence or absence of seed: phanerogams (visible marriage) and cryptogams (hidden marriage). In the former category were the seed plants, gymnosperms and angiosperms. In the latter category was a large phylogenetically diverse group of organisms including ferns, lycophytes, bryophytes, green algae, red algae, brown algae and fungi. Thus, cryptogams included what we now consider all those descendants from the primary endosymbiosis resulting in the chloroplast (except seed plants), some secondary endosymbionts (e.g. brown algae) and some phylogenetically unrelated eukaryotes (fungi). Considering this diversity of organisms, it is easy to imagine why it was difficult to generalize about cryptogams.

Discovery of sex in cryptogams

There is evidence that both Theophrastus and Pliny acknowledged sex in plants by stating that *'the fruit of the female date palm does not perfect itself unless the blossom of the male with its*

dust is shaken over it' and that *'naturalists tell us that all trees and even herbs have the two sexes'*. Like much of the wisdom of the Greeks, this thinking disappeared during the dark ages, with most during this time denying sex in plants. While the precise anatomical details of fertilization in angiosperms would not be known until improved microscopical techniques were developed in the 19th century, the basic tenets of sexuality and the functions of the floral organs were known in the 18th century. In contrast, the sexuality of cryptogams remained in a state of confusion, in large part due to attempts to construct analogies between the sexual organs of angiosperms with those of cryptogams. However, writing prior to 1545, Valerius Cordus (Cordus et al. 1561) clearly stated of the fern *Trichomanes*:

'It grows copiously on moist shaded rocks, although it produces no stem, or flower or seed. But it reproduces itself by means of the dust that is developed on the back of the leaves, as do all kinds of ferns; and let this statement of the fact once for all suffice.'
(Cordus et al. 1561) p. 170, transl. (Greene 1909)

He made similar statements regarding reproduction in *Aspidium* and *Phyllitis* (Cordus et al. 1561, Greene 1909). Thus, Cordus clearly distinguished the spores of ferns from seeds and also distinguished spores from pollen at a time when individual spores and pollen were not easily visible to the naked eye. Cordus' early death at age 29 prevented him from perhaps making more discoveries about the nature of cryptogamous plants.

Despite Cordus' writing, subsequently several authors made analogies between cryptogam spores and seeds (Hooke 1665, Browne 1672, Ray 1691, Morison 1699, Marchant 1713), while others equated spores with pollen (Micheli 1729, Dillenius 1741). The confusion derived from the lack of understanding of plant cycles, with many denying any sexual reproduction in cryptogams. Only when Casimir Christoph Schmidel and Johannes Hedwig correctly interpreted archegonia and antheridia as the female and male organs, respectively, was some clarity brought to the details of sexuality in bryophytes (Schmidel 1762, Hedwig 1783). Hedwig followed the entire life cycles of mosses and liverworts, using *M. polymorpha* as a model for the latter, conclusively demonstrating the functions of the sexual organs in the life cycle (Hedwig 1783). Both Schmidel and Hedwig also recognized vegetative reproduction via gemmae, contained within cups, or scyphules:

'If anyone meanwhile ponders the simplicity of parts, . . . which rules in the Umbels of each species, and the entirely wonderful artifice now exposed in the florets of the rays, which he carefully considers in each; with no difficulty will it agree with him, who has named the Umbels the seat of the masculine Organ, the Stars the dwelling place of the feminine, if also in that way the

Fig. 2 Continued

precision without artistic expression, Heinrich Fullmauer, who transferred the drawings to wooden blocks, and Veit Rudolf Speckle who cut the blocks (Arber 1912). Much to Fuchs' chagrin, his drawings were reused, often without acknowledgement, for the next two centuries. Particularly galling was the use of the images by publisher Christian Egenolph, who produced popular but inferior Herbals, of which Fuchs pointedly referred to their botanical mistakes, *'Among all the herbals which exist to-day, there are none which have more of the crassest errors than those which Egenolph, the printer, has already published again and again'* (Arber 1912).



Fig. 3 *Marchantia stellata*. Each frond stem [archegoniophore] of this plant (I) natural size, and (II) magnified, ends in a star or rosette one half inch in diameter and composed of nine rays. The underside of each of the beams (III) is lined with a plurality of membranes [perichaetum, perianth, calyptra] (a). Inside these membranes [perianth, calyptra] (b, c) resides a flower [sporangium] (d) that contains silky yellow-gold threads [elaters] (e) and infinitely small nearly round yellow particles [spores] (f). Marchant 1713; transl. J. L. Bowman and Pauline Jullien.

propagation of the plant may be more difficult; the most wise Creator will have provided for those progeny, sprouting within the scyphules . . .'

Schmidel (1783) p. 112, transl. Mark Garland

In the mid-19th century Wilhelm Hofmeister's recognition that all land plants possessed a spore-producing generation (sporophyte) and a generation bearing archegonia- and antheridia-producing gametes (gametophyte) provided the insight that united their life cycles and revolutionized subsequent botanical thinking (Hofmeister 1862). This clarified that while spores and seeds are both dispersal agents, they are produced in different generations, and that the similarity between spore and pollen development is due to their both being the formation of a gametophyte generation.

Plant animalcules

Motile cells of plants were discovered in the 1820s, and initially there was significant confusion as to their nature. They were sometimes called swarm cells as they tend to be produced in large numbers at once. Some regarded them as infusoria, animalcules or animals—a case of a plant being transformed into an animal, since plants were not supposed to move. Schmidel was the first to observe the discharge of spermatozoids in a liverwort, in *Fossombronia* (Schmidel 1783). In 1837, Franz Unger, who had previously reported motile sperm in *Sphagnum*, described the same in *M. polymorpha* (Fig. 4) and stated his conviction that the antherozoids were the male component of sexuality in mosses and liverworts (Unger 1837).

'Impatiently I awaited the first ripe antheridia of common Marchantiee (Marchantia polymorpha). Again, I found what I expected. That trapped in the same pollen-sacs [antheridia] behaved exactly as that of Polytrichum and other mosses; the fovilla [sperm] existed here properly in a cell mass, . . . but not only containing only cubic cells or more irregular graining, but all such of animal nature, as in the mosses. . . . I succeeded in Marchantia to observe the shape of the animals, which they take when swimming in the water, I could also perceive that the trunk, which now assumes a weak spiral position, and more so than when the rest of the body is stretched out, is undergoing a very rapid quivering movement.'

Unger (1837) p. 790–791, transl. J. L. Bowman

About the same time, Meyen also described 'sperm animals' from *Chara*, *Marchantia*, *Sphagnum* and *Hypnum* (Meyen 1838). Unger, Meyen and later Thuret (Thuret 1851) correctly portrayed the sperm with two anterior cilia.

Genetics of sex determination

The ancestral condition of the Marchantiophyta and of the Marchantiopsida (see Fig. 1) is predicted to be dioecy. Study of the genetic determination of sex in liverworts began soon after the rediscovery of Mendel at the beginning of the 20th century. Noll cultivated both male and female strains of *M. polymorpha* via gemmae for >30 generations and was unable to change their sexual character by subjecting them to various growth conditions (Schultze 1904, Blakeslee 1906). By

examining progeny, he concluded that the segregation of sex must take place during the maturation of the sporangium. Working with a related Marchantiopsida species, *Sphaerocarpus donnellii*, where the four products of meiosis remain attached to one another, Charles Douin definitively demonstrated that each tetrad of spores produced two male and two female gametophytes (Douin 1909). In 1917 Allen reported that female *S. donnellii* gametophytes have a large chromosome (X) for which the counterpart in male gametophytes was a small chromosome (Y)—the first report of sex chromosomes in plants (Allen 1917). Allen then confirmed Douin's observations and proposed that the reductive division of meiosis produced the segregation of the sex chromosomes and thus gametophyte sexuality (Allen 1919).

Occasional diploid gametophytes with a chromosome constitution of either $2A + 2Y$ or $2A + 2X$ have been identified and in these cases, the diploid gametophyte is fertile and of the sex the chromosome constitution suggests (Allen 1934, Allen 1935). Occasionally, spore dyads occur in *Sphaerocarpus*, the number varying depending upon the species (Lorbeer 1927, Knapp 1935). Typically the dyads have a chromosome constitution of $2A + X + Y$, suggesting non-disjunction during the first meiotic division. Diploid gametophytes derived from $2A + X + Y$ dyads give rise to morphologically female plants, indicating a dominance of the X over the Y in specifying sex (Lorbeer 1927, Allen 1935). However, the sexual organs can be intersexual, with some archegonia being functionally fertile, while others are morphologically similar to archegonia but with antheridia-like internal cell divisions (Lorbeer 1927, Allen 1935).

Once H.J. Muller demonstrated that X-ray irradiation was mutagenic (Muller 1927), this technique to induce mutations at will was applied to many organisms, including liverworts. Knapp irradiated *S. donnellii* spore mother cells prior to meiosis and noted that some tetrads produced one female and three males and some tetrads in which only three spores germinated, all of which were male (Knapp 1935). Cytological examination of these revealed that one of the males possessed an X chromosome from which variable portions had been lost. The males possessing an X chromosome were largely sterile with only weakly motile antherozoids. Knapp concluded that the *S. donnellii* X chromosome bears a gene or genes that promotes female development, but that males can be produced without a Y chromosome, although their sperm motility is compromised (Knapp 1936). While several X-linked mutations affected gametophyte development, Knapp argued that the majority of the heterochromatic X chromosome is dispensable for life.

Lorbeer also irradiated growing tips of female plants, from which a small proportion of regenerated shoots produced antheridia (Lorbeer 1936), but irradiation of males never resulted in a transformation into females (Lorbeer 1938). Irradiation of a female with $A + 2X$ chromosomes produced a few monoecious gametophytes producing archegonia, antheridia and intersex organs. When these were mated with a wild-type male, spores of the following generation produced fertile (motile antherozoids) males with an $A +$ mutated $X + Y$ chromosome constitution. Similar to Knapp, Lorbeer

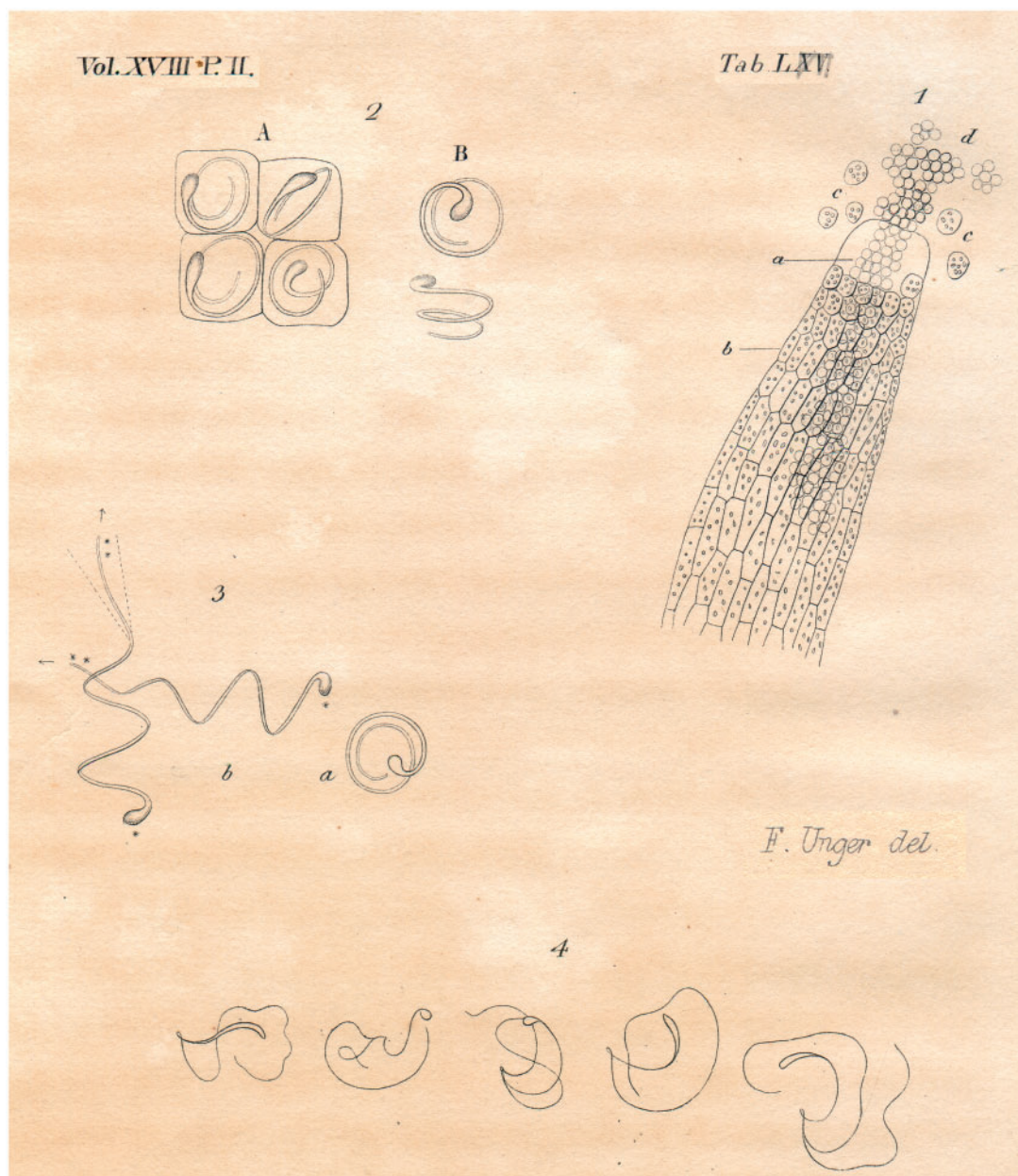


Fig. 4 Unger correctly represented the relationship of the two oscillating cilia at the fore end of the spermatozoon to the body at the posterior end. **Fig. 1.** The top part of a Pollinarium (antheridium) of *Polytrichum commune*. (a) Epidermis, (b) cell layer covering the content (fovilla) including, (c) individual cells separated from the bursting of the antheridium's tip, (d) fovilla, emanating in mass, consisting of very small cells that are a homogeneous slimy substance more or less intimately connected with one another. **Fig. 2.** (A) Close-up. You can see the cubic cells, whose side length is 0.0045, and in them the seed animals. (B) Those freed from the cell or membrane, have a diameter of 0.004. **Fig. 3.** Living seed animals of *Marchantia polymorpha*, (a) almost dormant, with contracted spiral, (b) drawn in the most lively movement; * body; ** trunk that oscillates in space. **Fig. 4.** On glass dried spermatozoa of *Marchantia polymorpha*, in different positions. From this we see the best view of the body length ratio contrasted with the thinner lengths' (Unger 1837); transl. J. L. Bowman.

postulated a Y chromosome 'mobilis' gene necessary for motile antherozoids and a female-promoting gene on the X, and since the latter remains active after loss of various parts of either arm, suggested that the female-promoting gene lies near the centromere (Lorbeer 1938, Lorbeer 1941). In addition, loss of portions of the X chromosome resulted in loss of vitality or even lethality if a Y chromosome is not present, suggesting the existence of an essential gene(s) residing on the regions shared by the X and Y, and that the shared region is essentially equivalent between the

sex chromosomes. While loss of chromosomes is lethal, the following constitutions were all observed to be viable: $A + X$, $2A + X$, $2A + 2X$ [gametophytic female]; $A + X + Y$, $2A + X + Y$ [gametophytic intersexual]; $A + Y$, $2A + 2Y$ [gametophytic male]; $2A + X + Y$, $3A + X + Y$, $3A + X + 2Y$, $3A + 2X + Y$, $4A + X + 2Y$, $4A + X + 3Y$ [sporophytic constitutions] (Mackay 1937, Allen 1945).

A combination of interspecific crosses and mutageneses suggest a scenario for *Marchantia* similar to that outlined for

Sphaerocarpus. Gertraud Haupt noted that the doubling of a sex chromosome (e.g. 2A + 2X and 2A + 2Y) does not influence phenotype in *M. polymorpha* (Haupt 1932). Likewise A + 2X and A + 2Y are morphologically normal fertile females and males, respectively. Furthermore, Haupt demonstrated that for both *M. polymorpha* and *M. planiloba* the female sex chromosome is dominant to the male sex chromosome in X + Y diploids, but that these diploids, while morphologically female, are sterile. Finally, observing the cytology of meiosis, she indicated that separation of the sex chromosomes takes place in the first meiotic division.

Hans Burgeff regenerated a few phenotypically female diploid gametophytes from the foot of *M. polymorpha* sporophytes, supporting the idea that the X chromosome is dominant over the Y (Burgeff 1937). He noted that spore dyads occasionally occur in *M. polymorpha* and *M. alpestris* and that they give rise to diploid gametophytes (2A + 2X and 2A + 2Y), in this case non-disjunction occurring during the second meiotic division. Matings between these diploids gives tetraploid, triploid and diploid sporophytes, the latter two classes presumably due to chromosome loss. If such losses typically occur at a high frequency, it may help explain the paucity of polyploidy in liverworts (Heitz 1927, Berrie 1960). Lorbeer also performed mutation experiments on *M. polymorpha* as described earlier for *Sphaerocarpus* (Lorbeer 1938). X-ray irradiation of *M. polymorpha* female plants resulted in a monoecious plant and a male plant, the latter with non-motile antherozoids, suggesting the conclusions outlined above for *Sphaerocarpus* may largely apply to *Marchantia*.

While dioecy is the norm in *Marchantia*, monoecy is 'not uncommon' in at least two species of *Marchantia*—*Preissia quadrata* [recent phylogenetic analyses indicate *Preissia* nests within the *Marchantia* genus (Forrest et al. 2006)] and *Marchantia wallisii*. It was first noted that dioecious Marchantiaceae species may occasionally be monoecious or androgynous by Taylor who described androgynous fructifications in *Dumortiera* (his *Hygrophylla irrigua*) in MacKay's *Flora Hibernica*: 'The fructification is commonly dioecious, sometimes monoecious and not very rarely androgynous as observed in *Marchantia androgyna* [synonymous with *P. quadrata*]. In this last case the anthers appear effaete and to have discharged their pollen long before the maturity of the seeds.' 'Specimens are not very uncommon in which the peduncle is truly androgynous, i.e. a part of the receptacle bearing capsules below, the rest bearing anthers on its superior surface.' (Taylor 1836). Androgynous receptacles in *P. quadrata* were also subsequently described by others (Goebel 1880, Leitgeb 1881, Townsend 1899, Haupt 1926, O'Hanlon 1927). However, it is not reported whether the antheridia of androgynous receptacles are functional.

Schuster suggested two subspecies of *P. quadrata*, a predominantly or wholly dioecious boreal-temperate type and an autoecious arctic type (Schuster 1992). Given the broad geographic distribution of *Preissia* and the loss of gemmae during the evolution of the *Preissia/Bucegia* lineage, the advantages of monoecy are obvious. In a *Preissia* population from New York, Arthur W. Haupt noted that about 1% of the thalli

were monoecious, but 20–30% had androgynous receptacles (Haupt 1926). The androgynous receptacles were mostly composed of two arms with antheridia and two with archegonia, the division being between the anterior and posterior lobes; only occasionally were there receptacles with one lobe of one sex and three of the other. The androgynous receptacles were reportedly fertile and have a split morphology as well as functionality, although some intersex organs, with attributes of both archegonia and antheridia, were observed. He further noted that there was a seasonality to the production of androgynous receptacles, with entirely male receptacles more common early in the breeding season and entirely female receptacles more common late in the season, and hence androgynous receptacles more common in mid-season (Haupt 1926). In Haupt's material, the plants had nine chromosomes, with one chromosome, probably the ancestral sex chromosome, much smaller than the rest. Depending on its identity, one scenario to explain the observations is a translocation of genes from either the X (female factor) or the Y (male mobility factor) to an autosome and their acquisition of seasonal regulation.

Gertraud Haupt described a unique chromosome constitution in *Marchantia grisea* (synonymous with *M. wallisii* and native to the Philippines and Sumatra), which has two types of gametophyte—male and monoecious but predominantly female (Haupt 1933). In the monoecious gametophyte, archegonia are produced in the typical manner for *Marchantia* species, but antheridia can develop almost anywhere on the dorsal surface of the thallus, even on the archegoniophore. Cytological analyses showed that the nuclei of the male gametophytes and the male cells of the monoecious gametophytes have nine chromosomes while the female regions of the monoecious gametophytes have an additional small heterochromatic 'z' chromosome (Haupt 1933). Haupt postulated that the presence of the z chromosome suppressed male development, resulting in female development and the production of archegonia. Loss of the z chromosome during mitotic divisions in the monoecious gametophyte would lead to male sectors producing antheridia. The z chromosome could also be lost during meiotic divisions, leading to the production of fully male gametophytes. This genetic system could have evolved via non-disjunction of the X–Y pair in an ancestor, the X evolving to become the 'z' chromosome and retained Y to provide motility genes.

The molecular era

In organisms where the diploid generation is dominant, the unique chromosome of the heterogametic sex undergoes degeneration due to accumulation of detrimental mutations and accumulation of heterochromatin, in a process first outlined by H.J. Muller (Muller 1914). In contrast, the evolutionary fate of sex chromosomes in organisms where the haploid generation is dominant is fundamentally different (Bull 1978). Three predictions of such sex chromosomes are: (i) the X and Y should have similar characteristics, with degeneration being similar for both; (ii) degeneration should be limited, with retention of genes required in the gametophyte and loss of genes required only

in the diploid; and (iii) changes in size should be additions of heterochromatin rather than losses.

The sequencing of the *M. polymorpha* Y chromosome provided support for only some of these hypotheses (Yamato et al. 2007). The chromosome is largely heterochromatic, harboring both genome-wide and Y-specific repetitive sequences. Only 64 genes were identified, of which 14 were male specific. Of the male-specific genes, some are expressed only in reproductive tissues and may encode proteins required for flagellar function—possibly the *mobilis* function of Lorbeer. Forty genes are expressed in both the gametophytic vegetative and male gametophore tissues, six of which have counterparts on the X that are expressed in the corresponding tissues in the female, and are therefore likely to have more general functions (Yamato et al. 2007). Genes having homologs on the X and Y chromosomes probably represent genes present on the ancestral autosome that gave rise to the sex chromosomes, implying retention of essential genes on sex chromosomes over an extended evolutionary time frame. Some of these genes have essentially saturated changes in the third position of the codons, indicating that they reside in regions that lack recombination with the X chromosome (Yamato et al. 2007). One aspect of theory that is not fully supported is the retention of genes (Bull 1978); the gene density on the Y chromosome is almost an order of magnitude less than that of the autosomes, implying extensive gene loss from the ancestral autosomal chromosome. Once an essential gene is translocated to an autosome, it can rapidly be lost on both sex chromosomes, leading to degeneration of both. Once the sequence of the X chromosome is determined, unresolved questions concerning whether the X chromosome has followed a similar evolutionary trajectory, the mechanism of pairing of the sex chromosomes, and if any recombination occurs, can be addressed.

The ancestral condition of the Marchantiopsida, and Marchantiophyta as a whole, is predicted to be dioecious, with the majority of extant species dioecious. Some species of the basal Haplomitriopsida lineage are reported to have heteromorphic chromosomes, and distinct sex chromosomes are found in most dioecious Marchantiopsida, with perhaps the exception of the basal-most lineage, the Blasiales (see Berrie 1960, Berrie 1963, and references therein). With the availability of the *M. polymorpha* sex chromosome sequences, their evolutionary trajectory can be explored within the Marchantiopsida and, more broadly, in the Marchantiophyta, to determine whether the extant sex-determining system of *Marchantia* is descended from an ancestral one that specified sex in the ancestral liverwort, or perhaps even land plants. Knowledge of the *M. polymorpha* sex-determining genes will also illuminate the variation observed within the genus. For example, in *M. grisea* is the 'z' chromosome descended from an ancestral X and in *Preissia*, if androgynous gametangiophores are both male and female fertile, has either the 'feminizing' locus or 'motility' genes normally residing on sex chromosomes been translocated to an autosome?

Within the Marchantiophyta, the ancestral liverwort may have possessed heteromorphic sex chromosomes and there is no evidence for ancient polyploidy (Berrie 1960). However, recent

polyploid species exist and polyploidy is often associated with monoecy (Heitz 1927). Monoecy, which has evolved multiple independent times within the Marchantiophyta, suggests that both the feminizing locus and motility genes are present in a single individual. Of particular interest in understanding the molecular genetic basis for the evolution of monoecy are species pairs consisting of a haploid dioecious species and a recently derived diploid monoecious species, as documented in the *Riccia fluitans* complex (Berrie 1964). Thus, the elucidation of the molecular genetic basis of sex determination in *M. polymorpha* should facilitate the understanding of the evolution of sex within the entirety of the Marchantiophyta, and perhaps other basal lineages of land plants.

Cell Theory and Mirbel's Mémoire

The first half of the 19th century saw a revolution in the understanding of plant development. Due to improvements in microscopy, and the widespread introduction of microscopes in which chromatic and, more importantly, spherical aberration had been reduced, the resolving power allowed the visualization of not only cellular but also subcellular detail. New observations stimulated much debate on both the nature of plant and animal tissues and their development. Major questions of the day were (i) is the entirety of an organism made of cells and (ii) where do new cells come from?

The first description of cells in plants was made by Robert Hooke whose interest in optics led him to examine a large number of items with his microscope, much of which is published in his *Micrographia* (Hooke 1665). Ironically, the first plant tissue he examined was petrified wood where he described 'conspicuous pores' that may have been vessels, resin canals, medullary rays or parenchyma cells (Baker 1948). At about the same time an English physician and botanist, Nehemiah Grew, initiated a study of plant anatomy, a short version of which was published in 1672 as *The Anatomy of Vegetables Begun*, and later as expanded into *Anatomy of Plants* (Grew 1682). Grew described the cellular nature of the cortex and pith and extended his observations to the cellular nature of seeds, allowing him to state '*this is the true texture of a plant, and the general composure, not only of a branch, but of all other parts from the seed to the seed*'. In Grew's descriptions, he uses Hooke's term 'cells', but also uses 'bladders', 'pores' and even 'bubbles', to refer to cells. The latter term, bubbles, was derived from Grew's erroneous notion that cells might be formed via fermentation, perhaps analogous to the bubbles in bread. A contemporary of Grew, Marcello Malpighi, is best known for his work on anatomy and embryology, but his work on plants in which he describes various elements comprising the plant body was published in *Anatome Plantarum* (Malpighii 1675). In this work he calls cells 'utriculi' and 'sacculi', a utriculus being a small bottle and sacculus a small bag, both of which imply holding liquid. Malpighi noted that if petals are broken up, rows of linked utriculi are released, foreshadowing a more accurate view of cells than implied by Grew's interpretations.

Charles-François Brisseau de Mirbel (1776–1854) published *Traité d'Anatomie et de Physiologie Végétale*, establishing his

place as founder of cytology, plant anatomy and plant physiology in France (Briseau-Mirbel 1802). Subsequently, in 1809, Mirbel published *Exposition de la Théorie de l'Organisation Végétale* wherein he described plants as consisting of cells forming a continuous membranous tissue, the 'tissu cellulaire' (Briseau-Mirbel 1809). As professor-administrator of the Jardin des Plantes in Paris, he turned his attention to *M. polymorpha*, to study the origin, development and organization of cellular structures (Fig. 5; Mirbel 1835). During these decades, Mirbel was embroiled in an argument with three German botanists (Treviranus, Link and Moldenhawer) about the nature of cells and cell formation.

With respect to the nature of cellular tissue, Mirbel was a disciple of Grew and Wolff, stating:

'The first idea, the fundamental idea is that all vegetable organization is formed by one and the same membranous tissue, variously modified. This fact is the base of all the others. The contrary idea is a source of errors. . . . Plants are composed of cells, all the parts of which are continuous among themselves; they present only one and the same membranous tissue.'

Briseau-Mirbel (1809) translated in Baker (1952)

In Mirbel's view, it was cellular tissue, and not the individual cell, that was regarded as fundamental, looking upon the organism as a cellular whole rather than an association of elementary unicellular organisms (Sharp 1934). In contrast, Treviranus and Link both thought that tissue consisted of cells that could be separated from one another (Treviranus 1805, Link 1807). Moldenhawer put the question beyond doubt using his technique of macerating tissue and observing that individual cells could be isolated, implying that the wall between two cells is a double wall, with each adjacent cell having its own wall (Moldenhawer 1812). This observation is at odds with a notion in which cells arise in a continuous membranous tissue whereby adjacent cells would share only a single common wall. Despite these observations Mirbel was reticent to change his views when communicating his initial results on *Marchantia* in 1831 and 1832 and only relented when the Memoire was finally published in 1835.

While Mirbel was initially entirely misguided about the cellular nature of tissue, he came closer to the correct view with respect to the origin of new cells. Treviranus, Link and others adhered to the idea that new cells 'crystallized' from Körner, granules or subcellular particles arising either inside of existing cells or in extracellular spaces. This idea that cells form in a process similar to crystallization would persist for some time, although the nature of the nucleating particle would change from a nondescript granule to the nucleolus to the nucleus over the span of the next few decades. In contrast, Mirbel believed that new cells arose from other pre-existing cells. In his Memoire on *Marchantia* he proposed three distinct mechanisms by which this could occur:

- (i) développement super-utriculaire, in which cells are produced at the surface of older cells;
- (ii) développement inter-utriculaire, in which cells are produced between adjacent walls of older cells;

- (iii) développement intra-utriculaire, in which cells are produced within older cells.

We now recognize, by a strict interpretation, that only one of these mechanisms, développement intra-utriculaire, occurs in plants and then, only in the specialized case of male gamete production. Also, in some species the formation of spores via meiosis may also be interpreted as développement intra-utriculaire. While Mirbel failed to observe the process of binary cell division—a single cell being divided into two, his développement super-utriculaire and développement inter-utriculaire could be describing this process, albeit incorrectly.

Mirbel's focus on développement intra-utriculaire may have been stimulated by his other work presented in the Memoire, that of the development of anthers and pollen in *Cucurbita pepo*, where the production of sperm cells in pollen is clearly a case of développement intra-utriculaire. In this regard it is of interest to note that both male and female gametophytes of flowering plants were often used as subjects for the study of cell formation, and both have rather unique patterns of cell formation from which generalizations cannot be made. For example, based on observations of embryo sac and endosperm development, Matthias Jakob Schleiden later generalized that new cells in plants were produced by cell-free formation, with a nucleolus first forming, then a nucleus, and then cytoplasm and eventually a cell wall (Schleiden 1838). While Schleiden is often given credit, in conjunction with Theodor Schwann and Rudolf Virchow, for the cell theory (Schwann and Schleiden 1847), it is clear that Schleiden's ideas on cell formation were in many regards no more accurate than Mirbel's. Between the time he presented his work to the Academie in 1831 and 1832 and its publication in 1835, Mirbel's views on cell formation evolved. By 1835 Mirbel no longer thought of organisms being composed of 'tissu cellulaire', but instead he consented they were made in their totality of utricules (cells). Furthermore, while the mechanisms he proposed were not correct in most details, he believed that new cells were derived from older cells. His work with *M. polymorpha* stimulated vigorous debate and by the middle of the 19th century questions regarding the cellular nature of plants and origins of new cells were finally settled.

Contemporaneous with Mirbel, the first clearly to document binary cell division was Barthélemy Charles Joseph Dumortier, who observed the process in an alga, *Conferva aurea*, in 1832 (Dumortier 1832), foreshadowing later studies demonstrating that this was the typical mode of new cell formation in both plants and animals. Dumortier's results were largely ignored, with later scientists rediscovering his results. For example, during the mid 1840s Unger, Mohl and Nägeli all formulated hypotheses of binary cell division being the primary mode of production of new plant cells, and it was Nägeli who finally demolished all vestiges of Schleiden's theories (Nägeli 1845, Nägeli 1846). In the course of analyzing cell division in bryophytes, Nägeli described that apical cells were responsible for producing both shoots in mosses and leafy liverworts, and also provided a detailed description of cell division during gemma development in *Lunularia* (Nägeli 1845). Nägeli's was the first

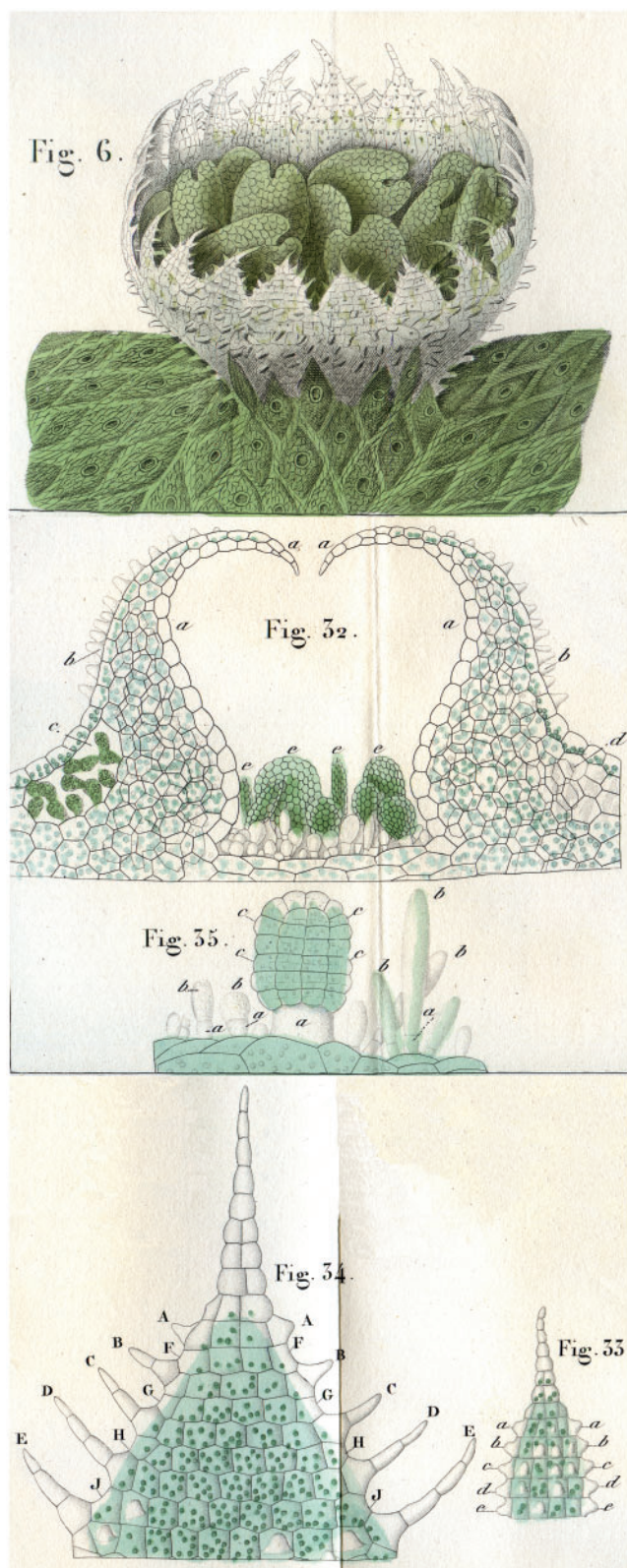


Fig. 5 Two of the mechanisms by which Mirbel proposed that new cells could form which he described in gemmae and their cups. He hypothesized that new cells arose on the surface of older cells, 'développement super-utriculaire', as he thought he observed in the formation of teeth at tips of gemmae cups, or alternatively, cells could form on the inner walls of old cells, 'développement intra-utriculaire',

description of apical cells in plants, spurring a search for such cells in other plants.

The exquisite drawings of *M. polymorpha* in Mirbel's Memoire were drawn by Mlle. F. Legendre, a relative of Mirbel's and said to be the best botanical artiste in Paris at the time, and engraved and colored under the direction of P. Duménil [Figs. 5–6; (Gray 1894)]. Mirbel believed, as did many others at the time, that more accurate representations would be obtained, and preconceived prejudices eliminated, if the observer and writer did not produce the illustrations (Sachs 1890). The drawings are notable for both the detail they provide and also what they lack. For example, subcellular details are included, with chloroplasts depicted in several plates, despite the fact that the function of chloroplasts was not worked out until the second half of the 19th century. In contrast, a subcellular detail notably absent in all of Mirbel's illustrations is the nucleus, which was first described in detail in 1833 by Robert Brown (Brown 1833), and of whose work Mirbel may not have been aware. While Mirbel was not ultimately successful in answering the questions he initially proposed, he firmly established that *M. polymorpha* was a tractable model organism in which to address fundamental biological questions.

Establishment of Polarity in Gemmalings

The gemmae of *M. polymorpha* furnished one of the first systems in which the factors influencing the establishment of organismal polarity were identified. While his memoire focused on the origin of new cells, Mirbel may have been the first to inquire experimentally as to the nature of the establishment of polarity in plants (Mirbel 1835). While developing within the cup, gemmae are apolar with respect to their dorsiventral axis, and remain in stasis until displaced from the cup (Schröder 1886, Molisch 1922, Oppenheimer 1922, Tarén 1958). The cup acts as a splash cup aiding in the dispersal of gemmae by raindrops (Kny 1890, Brodie 1951, Equihua 1987). After displacement from the cup, dorsiventral differentiation depends upon the position of a gemma with respect to external environmental factors, the first of which was identified by Mirbel as light (Fig. 6).

Fig. 5 Continued

which he thought he observed during the development of gemmae. **Fig. 6** Gemmae cup containing elliptical lenticular bulbils [gemmae] notched on both ends. These small baskets, with serrated edges, are found on the fronds. Fig. 32. Longitudinal section of young gemmae basket. (a) Teeth surrounding the basket. (b) Superficial cells with conical nipples. (c) Air chamber and papillae. (d) Air chamber viewed through the utricular tissue. (e) Clusters of bulbils [gemmae], all very young, but at different stages of development. Fig. 33. A tooth of a basket. Fig. 34. A tooth from an older basket like that shown Fig. 6. Fig. 35. Very young bulbils, observed by a magnification of 500–600×. (a) Utricle forming the bulbil peduncle. (b) Utricle that serves as template for a bulbil. (c) Bulbil shortly after the absorption of the utricule in which it is developed'. (Mirbel 1835); transl. J. L. Bowman and Pauline Jullien.

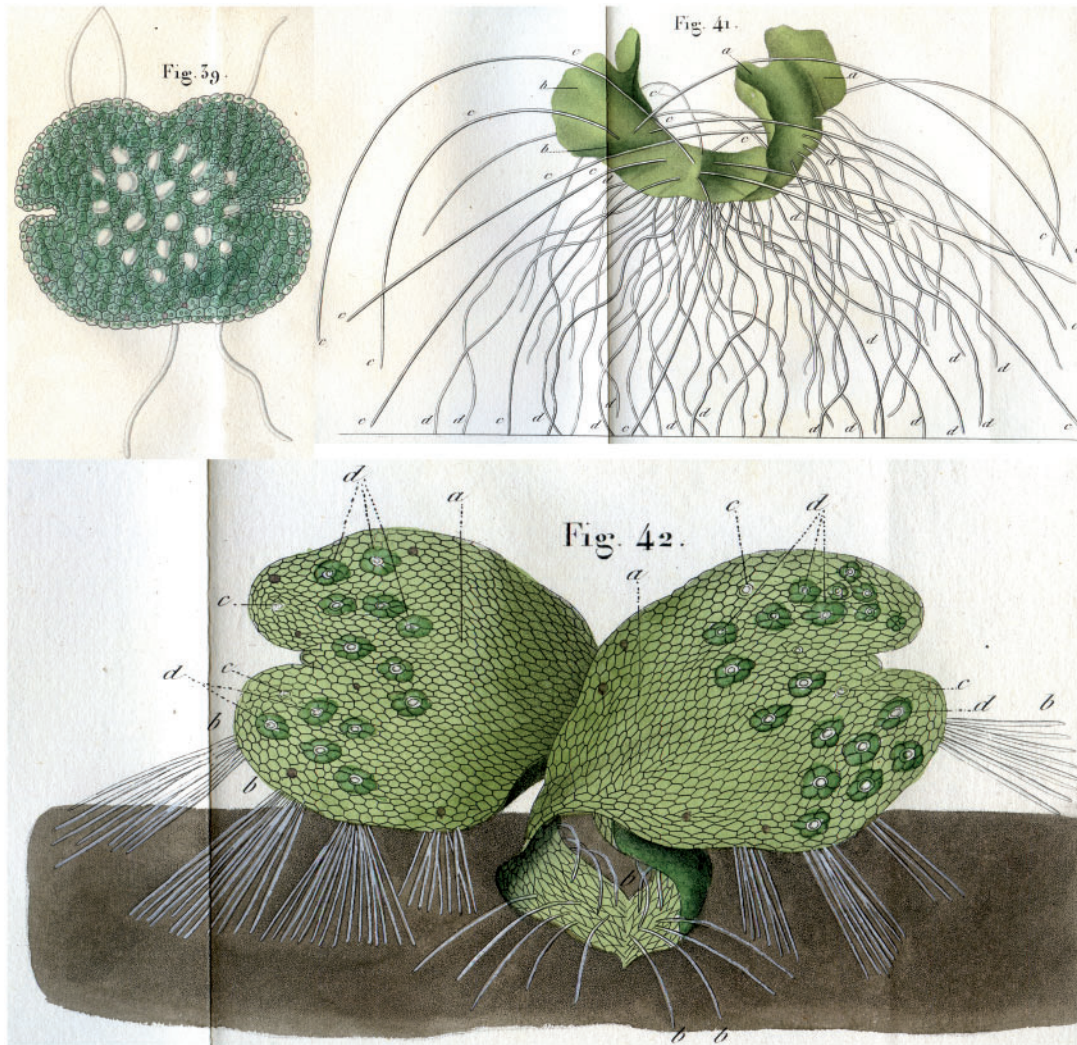


Fig. 6 Mirbel's experiments on establishment of polarity in gemmalings (Mirbel 1835). See text for description.

'One morning I laid flat on powdered sandstone, many bulbils [gemmae] (39). The next day, at the same time, I turned all over (40). So there was exchange of position between the upper and the lower surfaces that I will continue to call as they were originally, despite the reversal. Twenty-four hours had sufficed for the lower surface to produce several rhizoids, some of which had a considerable length, and though this surface was then exposed to air and light, these roots continue to elongate and project in an arc with their free end towards the soil (41c). For its part, the upper surface produced many roots, especially the middle part. (41d).

However bulbils continued to grow. In a few days I saw successively the two opposing lobes, which, initially, were applied to the ground, lift, stand and bow their vertices inside, stand to meet the other, deflect a little of their original direction, one right, one left, as if to make way, mingle, and finally cross (42). The consequence of this development . . . was that the top surface is found, if not in whole, at least in large part, towards the sky, despite the reversal to which I had subjected it, and soon was covered with stomata (42a,d).

The lower surface after the reversal, produced no stomata, and even in places that light directly hit, produced many roots when it found itself in the shade and moisture after the twisting growth (42a), and produced an embossed midline.' (Mirbel 1835); transl. J. L. Bowman and Pauline Jullien

From these experiments Mirbel stated that light induced polarity, with the side that was towards the light differentiating as dorsal and the side towards the ground developing as ventral. In his experiments this happened within 24 h, and he demonstrated that, once induced, it is permanent—if the thalli were turned over they would grow in a twisted manner such that the original dorsal surface was again oriented towards the light.

A half century later, Wilhelm Pfeffer noted some variability in the growth of *M. polymorpha* gemmae, a feature that resulted in sometimes contradictory reports by subsequent workers. Pfeffer demonstrated the requirement for light and water in gemma germination (Fig. 7). He described rhizoids emerging after, on average, 1.5–2 d, establishment of irreversible polarity in 2–3 d (in contrast to Mirbel's 24 h), and formation of air chambers after 10–14 d in the light, thus providing the first evidence that the three processes can be developmentally separable events, and that polarity induction occurs in very young apices prior to anatomical differentiation (Pfeffer 1871). Pfeffer germinated gemmae in uniform diffuse light and observed slow shoot growth and substantial rhizoid growth from the side

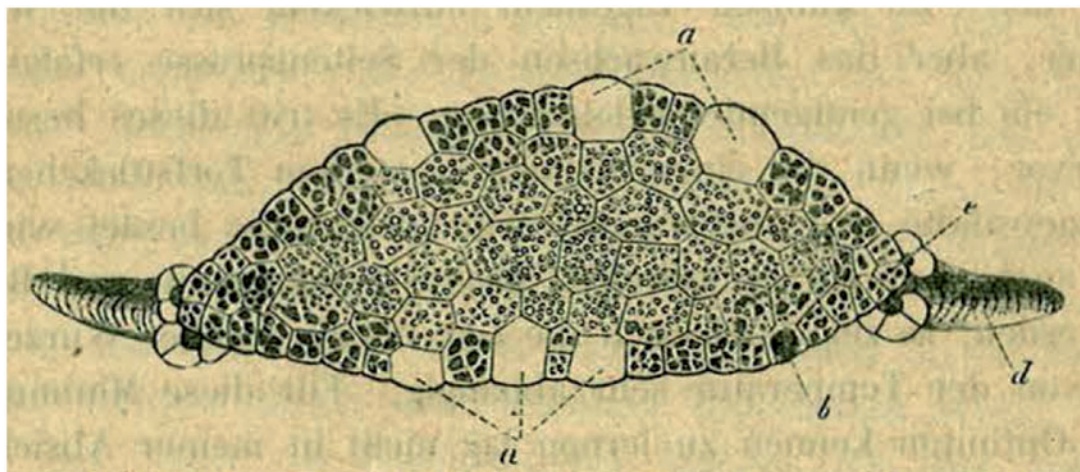


Fig. 7 Section perpendicular to the surface and through the two growing points of a mature gemmae of *Marchantia polymorpha*; (a) the hyaline rhizoid cells; (b) oil body cells; (d) wings flanking the growing points (e) (Pfeffer 1871).

towards the earth, leading him to suggest that gravity might promote rhizoid growth on the side towards the earth (Pfeffer 1871). He initially reported that contact with a substrate also induced rhizoid growth, but later recanted this conclusion, instead concluding that substrate moisture was not involved in initial rhizoid emergence, but only later in subsequent rhizoid growth (Pfeffer 1885).

A decade later Zimmerman grew *M. polymorpha* gemmae floating on water with illumination from either above or below, using a south-facing window and mirrors to redirect the incoming light (Zimmerman 1882). When illuminated from below, rhizoids formed predominantly from the upper surface, while control gemmae developed rhizoids predominantly on the lower sides. He noted that in plants experiencing intense illumination from below, air pores were formed facing the water surface with rhizoids extending into the air, highlighting the critical role of light in polarity establishment (Zimmerman 1882). Leitgeb had reported similar results with germinating *M. polymorpha* sporelings—if illuminated from below, the lower surface developed air pores and chambers (Leitgeb 1877). The first to attempt to provide uniform environmental conditions to growing *M. polymorpha* gemmae was Czapek, who placed gemmae in uniform light on a clinostat. He observed small tubular thallus lobes with rhizoids all around, which he interpreted as isolateral gemma growth, an observation that foreshadowed experiments in the 20th century (Czapek 1898).

Beauverie, working with both *M. polymorpha* and *Lunularia cruciata*, also noted permanence of polarity (Beauverie 1898). He transferred adult thalli from normal conditions into feeble uniform light and observed that the originally horizontal thalli grew vertically, presumably due to negative geotropism. The vertical thalli were light green, narrow and curled up towards the dorsal side, with few air pores and air chambers (lacking filaments) and with chlorophyll present on both dorsal and ventral sides with near equal intensity. However, the original ventral surface never produced air chambers and the original dorsal surface never produced rhizoids or scales (Beauverie 1898).

The reports in the 19th century strongly implicated light as a critical factor and invoked gravity as an influence. However, in these early reports variability was noted both in the timing of events and in the universality of dorsiventral orientation, with a fraction of gemmalings differentiating contrary to what was expected. Likewise, in the early part of the 20th century, Dacknowski also noted variability in the development of gemmalings, and Förster described development of some isolateral gemmalings when gemmae germinated floating on a liquid medium (Dacknowski 1907, Förster 1927).

In an exhaustive series of experiments during the 1930s, Hans Fitting, working at the University of Bonn, explored the causes of variability, concluding that the sensitivity of gemmae to environmental conditions is influenced by the environment the parent thallus experiences. Fitting first repeated the experiments of previous researchers and noted isolateral *M. polymorpha* gemmalings when he illuminated germinating gemmae from below, probably resulting in relatively uniform lighting (Fitting 1936a). He described them as being 'winged' and having rhizoids all around the outer surfaces, with dorsal tissues being produced in the notch created by the growth of each of the two wings at each end of the gemmaling, and that isolateral gemmalings could be reproducibly produced by growth on a clinostat with even illumination (Fitting 1936a). By comparing gemmae germination with light from above, or, alternatively, illuminated from below, he obtained results that varied with season—gemmalings germinating in winter behaved as if light was the most important factor, but gemmalings germinating in June did not strictly follow the light regime. Fitting noted that *M. polymorpha* gemmae did not germinate in the dark—after 60 d either none or only a few rhizoids developed from dark-grown gemmae on nutrient agar (Fitting 1936b). By providing a geoinduction in the dark prior to growth on the evenly illuminated clinostat, he could show that a geoinduction as short as 6 h, but more reliably 12.5–16 h could induce dorsiventrality without unilateral illumination, indicating that ungerminated gemmae are sensitive to gravity and that in the absence of a unilateral light signal, geoinduction could suffice for polarity establishment (Fitting 1936b). In a similar set of experiments,

gemmae were given a unilateral photoinduction of a short period of time while placed on a clinostat and then grown on the clinostat with uniform illumination. Fitting found that as little as 5 h of photoinduction was sufficient to induce polarity establishment (Fitting 1937).

In all of his experiments, Fitting observed some variability in response; for example, very young gemmae from the bottom of the cup were less sensitive than older gemmae from the top of the cup. When thalli were grown on the clinostat while gemmae were developing within the cups, these gemmae produced very few isolateral gemmalings, suggesting that conditions within the cup can influence later development. Again he noted variability correlated with the seasons and introduced the concept of the 'mood' of the gemmae that varied seasonally, e.g. winter ('dark-mooded') gemmae behave differently as compared with summer ('light-mooded') gemmae (Fitting 1937). Under bright light, winter gemmae almost always develop with their dorsal side towards the light, whereas summer gemmae sometimes do not, suggesting that winter gemmae are more responsive to light than are summer gemmae. Furthermore, the times at which photo- and geoinductions of dorsiventrality are irreversibly stabilized varies seasonally (Fitting 1938). Fitting found that by using environmental treatments, e.g. uniform illumination for 36 h, dark-mooded winter gemmae could be converted into light-mooded summer gemmae. Fitting suggested that the variability in the observations of previous researchers is due to the 'mood' of the gemmae, and he speculated, for example, that Mirbel used summer gemmae and performed experiments in bright sunshine while Pfeffer used winter gemmae under weaker artificial lighting (Fitting 1938, Fitting 1939). Fitting proposed that in natural conditions, summer gemmae, due to their exposure to bright sunshine, can acquire some dorsiventrality when they are still in the cup, with their variable position within the cup (e.g. somewhat horizontal if the cup is full and it has not rained for some time) influencing subsequent development once they are displaced. In contrast, winter gemmae are more labile than summer gemmae, with any dorsiventrality induced within the cup easily reversed once they are displaced, with subsequent unilateral illumination and gravity dictating polarity establishment (Fitting 1938). Consistent with Pfeffer's results Fitting found that rhizoid development could be triggered in about 2 h, induction of dorsiventral polarity in about 8 h, the polarity stabilized in about 16 h, and that >30 h was required for the commencement of gemmae growth (Fitting 1939). Finally, foreshadowing the next few decades of experiments, Fitting demonstrated that exogenous application of β -IAA could induce winter gemmae to develop rhizoids and initiate gemmae growth in the dark (Fitting 1939).

While Fitting was performing the experiments described above, his student, Wilhem Halbsguth, examined the anatomy of the shoot apices of developing gemmalings. Halbsguth found that in 20 (25%) of the 83 *M. polymorpha* apices he examined only a single apical cell was visible, while 37 (44%) had two potential apical cells, and 26 (31%) had two conspicuous apical cells [Fig. 8 (Halbsguth 1937)]. When he examined apices after 80 h of unilateral light and gravity stimulation, he

observed apices in which the ventral gemma apical cell differentiated into a thallus apical cell, while the dorsal gemma apical cell remained dormant or developed only a little (Halbsguth 1937). In contrast, when he examined gemmalings after 60 h growth on a clinostat with uniform illumination, he observed that both the ventral and dorsal gemma apical cells had commenced divisions indicative of their both establishing thallus apical cells (Halbsguth 1937). Halbsguth proposed that *M. polymorpha* gemmae are inherently isolateral and that upon unilateral stimulation the ventral gemma apical cell develops into a dorsiventral thallus while the dorsal gemma apical cell remains quiescent and gets displaced dorsally on the developing thallus. In contrast, under uniform light and gravity stimulation, both gemma apical cells have the potential to develop into a thallus, creating 'winged' or isolateral thalli (Halbsguth 1937). In the early 1960s, Morton Miller, Andrew Sparrow and colleagues carried out experiments measuring the sensitivity of *M. polymorpha* gemmae to acute gamma radiation and concluded that apical cell number was approximately 2, supporting Halbsguth's idea that each gemmae apex has two apical cells (Miller and Alvarez 1965, Miller 1966).

Following The Second World War, Halbsguth and his students continued work on *M. polymorpha* gemmae, following up Fitting's observation with respect to the inductive capacity of auxin. They demonstrated that exogenous IAA increased the percentage of isolateral gemmalings when grown on a clinostat with uniform illumination and that the effects of dark geoinduction prior to growth under uniform illumination is reduced by exogenous IAA (Halbsguth and Kohlenbach 1953). In contrast, exogenous IAA appeared to increase the effectiveness of a suboptimal photoinduction. Based on these observations, it was proposed that IAA acts as a genuine growth substance involved in directing the establishment of dorsiventrality in *Marchantia* gemmalings (Halbsguth 1953, Halbsguth and Kohlenbach 1953). Consistent with Fitting's 'moods', Halbsguth noted that the growth conditions of the parent thallus, i.e. conditions of the gemmae within the cup, can influence a subsequent geoinduction. Halbsguth's student, Kohlenbach, noted that soaking gemmae in a low concentration of exogenous IAA for 24–48 h previously can enhance a dark geoinduction (Kohlenbach 1957). This effect was especially noticeable when winter gemmae were used—exogenous auxin could result in effective geoinductions in a time course that was otherwise insufficient. Conversely, soaking in a high concentration of exogenous IAA could weaken the effects of a dark geoinduction. Kohlenbach concluded that *M. polymorpha* gemmae must possess an endogenous growth substance (presumably auxin although at this time it had not yet been isolated from *Marchantia*) and that external factors that direct the dorsiventrality create within the gemmae an auxin gradient (Kohlenbach 1957). He further speculated that the difference between winter and summer gemmae is partly due to their differing auxin content (Kohlenbach 1957).

In the first half of the 1950s, Jacqueline Rousseau also examined the effects of exogenous auxin on the emergence of rhizoids and growth of gemmae. She found that high concentrations killed gemmae but lower concentrations

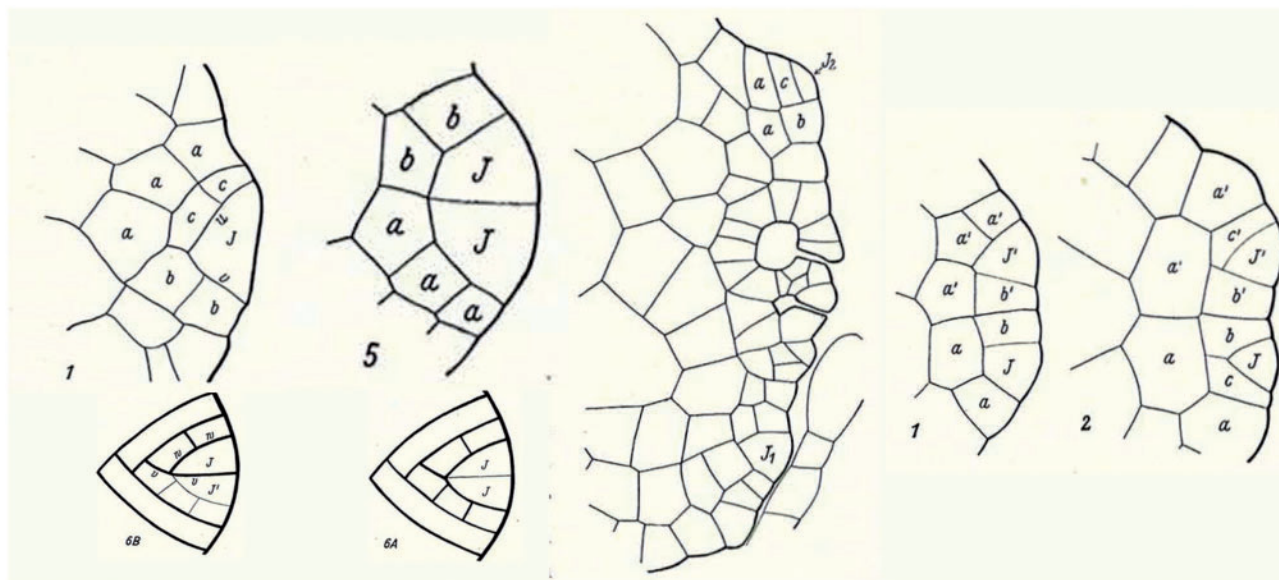


Fig. 8 Left panels 1, 5 and 6: *Marchantia polymorpha* gemma apices with only a single (1-left) or two (5) apical cells. Diagrams illustrating the equality in appearance with real (6A) and 'apparent' (6B) dichotomies. Middle panel: gemmaling apex after 80 h unilateral light and gravity stimulation. Right panels 1 and 2: gemmaling apices after 60 h growth on a clinostat with uniform lighting. Reprinted with permission from Halbsguth 1937 (Schweizerbart Science Publishers, www.schweizerbart.de).

resulted in dorsal rhizoid formation (Rousseau 1950). Rousseau also repeated Fitting's experiment, demonstrating that exogenous auxin (β -IAA) or auxin analogs [e.g. β -indole propionic acid, β -naphthoxyacetic acid, 2,4-dichlorophenoxyacetic acid or α -naphthalene acetic acid (NAA)] could stimulate *M. polymorpha* gemmae to begin their development in the dark, and concluded that auxin can compensate for light (Rousseau 1954), a similar conclusion to that which Fitting had arrived at with respect to spore germination (Fitting 1939). Due to the auxin sensitivity of the emergence of rhizoids of *M. polymorpha* gemmae, Rousseau and Schutzenberger proposed they could be used as an assay for auxin, similar to the *Avena* test (Rousseau and Schutzenberger 1952). By comparing the number and density of rhizoids on gemmae grown in the presence of varying concentrations of α -NAA, a dose–response curve could be generated. However, the curve was parabolic, being complicated by the toxic effects of α -NAA at higher concentrations so that the peak in rhizoid number was at intermediate concentrations, with the frequency falling off at higher and lower concentrations (Rousseau and Schutzenberger 1952). Despite its sensitivity, the assay was not taken up by the general community. Experiments applying exogenous auxin, auxin analogs and anti-auxins to *Marchantia* gemmae continued to be repeated for the next few decades, but little more insight was gained (Moewus and Schader 1952, LaRue and Narayanaswami 1954, Tarén 1958, Maravolo and Voth 1966, Allsopp et al. 1968, Prior and Brown 1970).

While some early attempts to isolate auxin from *Marchantia* were unsuccessful (Goeddecke 1935), Löbenberg succeeded in isolating IAA from *M. polymorpha* gemmae and thalli utilizing paper chromatography after extraction (Löbenberg 1959), and Karl Fries isolated IAA from *M. polymorpha* thalli (Fries 1964).

Fries found that the IAA concentration varied with the age of the tissue—it was readily isolated from 14-day-old gemmalings, but was undetectable in ungerminated gemmae (Fries 1964). Schneider et al. (1967) also reported the isolation of IAA from *M. polymorpha*.

Students of Halbsguth continued to investigate aspects of gemmae development. Karl-Ronald Otto revisited the exogenous and endogenous factors that influence rhizoid emergence (Otto 1976, Otto and Halbsguth 1976). He first re-examined the effects of light, gravity and substrate on *M. polymorpha* gemma rhizoid emergence. Gemmae were plated in the dark or after light treatment for 1 h, 24 h and 4 d, either on the clinostat or not, and gemmae were examined at age 12 d. He found that in the dark, up to 20% of gemmae produced ventral rhizoids but no dorsal rhizoids, consistent with a role for gravity and with the variability attributed to the differing 'moods' of the gemmae as earlier noted by Fitting (Otto 1976). If grown on the clinostat in darkness, rhizoid emergence was suppressed compared with the control, but if gemmae were unilaterally illuminated with red light for 1 h, most (70–90%) produced ventral rhizoids, but no dorsal rhizoids emerged. If the red light illumination was extended for 4 d, all gemmae produced both ventral and dorsal rhizoids. Rhizoid emergence was dependent upon the wavelength of illumination, with 650 nm (red) most effective and irradiation of shorter than 550 nm or longer than 670 nm not effective (Otto and Halbsguth 1976). The activity of red light illumination could be reversed with far-red illumination, implicating phytochrome as an effector of rhizoid emergence (Otto and Halbsguth 1976). Otto concluded that gravity effects rhizoid emergence, and that light, especially red light, could stimulate ventral rhizoids in the presence of gravity and both dorsal and ventral rhizoids in the absence of gravity (Otto

1976). He then kept gemmae in the dark for 12 h prior to a 6 h red irradiation. If gemmae were kept in their original dorsiventral position, rhizoids only emerged from the ventral surface, whereas if the gemmae were inverted just prior to the irradiation, all gemmae formed both dorsal and ventral rhizoids. Thus, the red illumination stimulated rhizoid emergence from both the previous and new gravitationally stimulated surfaces (Otto 1976). Since light cannot fully induce rhizoid emergence on its own, Otto proposed that light also acts as an orienting factor, with its effect stronger at the surface facing away from the light, implying that light induces an internal gradient opposite to its own intensity (Otto 1976). As a mechanism to establish the hypothesized gradient, Otto suggested: 'It is conceivable that light causes the production or activation of a rhizoid growth inducing substance which is then transported away from the irradiated surface.' (Otto 1976). Since emersion of gemmae in a 10^{-4} M solution of IAA provides a similar stimulus for rhizoid emergence as a 1 h red light illumination, Otto and Halbsguth suggested that phytochrome activity may be linked to membrane permeability of IAA, in accordance with the transport model hypothesized by Otto (Otto and Halbsguth 1976).

A synthesis of results from experimental morphology experiments over the last two centuries leads to a model whereby dorsiventral polarity is established in stages as outlined by Pfeffer and Fitting—emergence of rhizoids after 2–3 h, followed by induction of dorsiventral polarity (~5–8 h) and its subsequent stabilization (16 h), and then finally initial growth of the gemmae apices (~30 h). The initial formation of rhizoids is mediated by localized auxin synthesis and/or transport away from the light source, and is mediated by phytochrome activity. The emergence of rhizoids helps fix the gemmae to the substrate. The establishment of dorsiventral polarity can be induced by light and/or gravity and is likely to also involve localized auxin synthesis and/or transport, leading to the continued activity of the ventrally located apical cell in the gemma meristem and the eventual suppression of the dorsal apical cell of the gemma meristem. Once stabilized, the polarity is permanent, implying that polarity is imposed upon dorsal and ventral derivatives of the apical cell, perhaps via signaling from adjacent differentiating or differentiated tissues. Before the bicentennial of Mirbel's experiments, the tractability of *Marchantia* as a model genetic system should allow the designation of specific gene and protein activities to the experimental morphology of the past.

Epilogue

While only a small fraction of the literature available on *Marchantia* and its Marchantiopsida relatives can be covered in a short review, much of the older literature is becoming freely accessible through resources such as the Biodiversity Heritage Library, which has >10,000 entries on *Marchantia*. It is hoped that as *Marchantia* enters the genomic era we can draw on the rich historical literature, standing on the shoulders of our predecessors who have provided a foundation to further our

understanding not only of *Marchantia*, but of the evolution, development and physiology of land plants, whose terrestrial conquest altered the course of our planet.

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