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VIEWPOINT

The origin of the sporophyte shoot in land plants: a bryological perspective

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• *Background* Land plants (embryophytes) are monophyletic and encompass four major clades: liverworts, mosses, hornworts and polysporangiophytes. The liverworts are resolved as the earliest divergent lineage and the mosses as sister to a crown clade formed by the hornworts and polysporangiophytes (lycophytes, monilophytes and seed plants). Alternative topologies resolving the hornworts as sister to mosses plus polysporangiophytes are less well supported. Sporophyte development in liverworts depends only on embryonic formative cell divisions. A transient basal meristem contributes part of the sporophyte in mosses. The sporophyte body in hornworts and polysporangiophytes develops predominantly by post-embryonic meristematic activity.
• *Scope* This paper explores the origin of the sporophyte shoot in terms of changes in embryo organization. Pressure towards amplification of the sporangium-associated photosynthetic apparatus was a major driver of sporophyte evolution. Starting from a putative ancestral condition in which a transient basal meristem produced a sporangium-supporting seta, we postulate that in the hornwort–polysporangiophyte lineage the basal meristem acquired indeterminate meristematic activity and ectopically expressed the sporangium morphogenetic programme. The resulting sporophyte body plan remained substantially unaltered in hornworts, whereas in polysporangiophytes the persistent meristem shifted from a mid-embryo to a superficial position and was converted into an ancestral shoot apical meristem with the evolution of sequential vegetative and reproductive growth.
• *Conclusions* The sporophyte shoot is interpreted as a sterilized sporangial axis interpolated between the embryo and the fertile sporangium. With reference to the putatively ancestral condition found in mosses, the sporophyte body plans in hornworts and polysporangiophytes are viewed as the product of opposite heterochronic events, i.e. an anticipation and a delay, respectively, in the development of the sporangium. In either case the result was a pedomorphic sporophyte permanently retaining juvenile characters.

Key words: bryophytes, embryo, development, meristems, plant evolution, sporophyte shoot, stomata.

INTRODUCTION

Molecular phylogenies have resolved land plants (embryophytes) as monophyletic with charophytic ancestry. Living land plants encompass four major clades: liverworts, mosses, hornworts and tracheophytes (lycophytes, monilophytes and seed plants). The liverworts are resolved as the earliest divergent lineage and the mosses as the sister group to a crown clade formed by the hornworts and tracheophytes. Alternative topologies resolving the mosses as the sister group to tracheophytes are less well supported (Qiu *et al.*, 2006, 2007; Qiu, 2008; Chang and Graham, 2011).

Characterized by a dominant gametophyte and uniaxial sporophyte permanently associated with the gametophyte, liverworts, mosses and hornworts are traditionally referred to as 'bryophytes', a taxonomic assemblage now considered paraphyletic. Bryophytes produce sporophytes with a single sporangium or capsule, hence the designation as monosporangiates. The tracheophytes are markedly different from bryophytes in that after an initial embryonic phase the sporophyte becomes autonomous, ramifies and produces multiple sporangia. Extant and extinct tracheophytes, plus some fossil relatives with branched sporophytes but possibly lacking lignified vascular tissue, are collectively referred to as the polysporangiophytes (Kenrick and Crane, 1997*a*, *b*; Kenrick, 2000; Gerrienne and Gonez, 2011).

Microfossil evidence indicates that land plants with bryophytic affinities appeared in the Ordovician at least 470 million years ago. This is consistent with an estimation of the divergence time of liverworts, the earliest extant land plant lineage, suggesting a Late-Ordovician origin (Heinrichs *et al.*, 2007). The oldest accepted land plant macrofossils are from Mid-Silurian rocks with an age of about 425 million years and have been described as isotomously branched sporophyte axes bearing terminal *Cooksonia*-type sporangia (Edwards and Feehan, 1980). Macrofossils recognized as zosterophylls (e.g. *Bathurstia* and *Zosterophyllum*; Kotyk *et al.*, 2002) or basal lycopsids (eg *Baragwanathia*; Richards, 2000), both relatively advanced members of the tracheophyte lineage (Kenrick and Crane, 1997*a*, *b*), have been described from Late-Silurian compressions about 410 million year old. Thus, although sparse and somewhat controversial, the paleobotanical evidence indicates that the transition from the bryophyte grade to the polysporangiate grade took place during a

45 million year interval between the Mid Ordovician and Mid Silurian. Molecular clock analyses suggest earlier origins for major embryophyte clades (e.g. 568–815 million years ago in Clarke *et al.*, 2011), possibly implying a longer time for the bryophyte to polysporangiophyte transition.

The mature sporophyte of liverworts consists of a sporangium or capsule, containing the spore-forming apparatus, a seta elongating solely by cell expansion (Thomas, 1980), and an absorptive foot. This body plan is established by formative cell divisions (Gunning *et al.*, 1978) at an early stage of development; subsequent sporophyte growth depends on proliferative cell division and cell expansion in the absence of any localized area of cell division recognizable as a meristem (Cooke *et al.*, 2004). The mature sporophyte in mosses has a similar anatomy to that in liverworts, but the seta and a part of the foot arise from a transient meristem developing in the middle of the spindle-shaped embryo. The mature sporophyte of hornworts lacks a seta and consists of a foot and a sporangial axis, the latter growing from a basal meristem that remains active throughout sporophyte life (Cooke *et al.*, 2004; Sakakibara *et al.*, 2008; Ligrone *et al.*, 2012). The sporophyte body plan of polysporangiophytes, in its basic form epitomized by leafless rhyniopsid plants (Taylor *et al.*, 2009), is a free-living branched axial body (the sporophyte shoot) developing from a persistent apical meristem (the shoot apical meristem, or SAM) and eventually producing multiple terminal sporangia. In the following discussion, the term sporophyte shoot will be used to indicate the vegetative part of the sporophyte in polysporangiophytes, independent of the presence of leaves. We define the embryo as the post-fertilization developmental stage during which the sporophyte body plan is established. In liverworts this coincides with the phase of formative cell division. The same does not hold true for mosses, hornworts and polysporangiophytes because here, owing to the development of a meristematic area, formative cell divisions persist after the establishment of the sporophyte body plan. For these three groups, we view the appearance of a meristem (either basal or apical) as the event marking the transition from embryonic to post-embryonic sporophyte development.

Despite profound differences in ontogeny, the sporophyte shoot in polysporangiophytes has generally been assumed to have evolved from the seta of mosses (Smith, 1955; Mishler and Churchill, 1985). Rejecting this view, Kato and Akiyama (2005) interpreted the seta as part of the bryophyte sporangium, and the sporophyte shoot as a novel structure interpolated between the embryo and sporogenesis. This appears to overlook the fact that evolution is a contingent process that produces innovations by modifying already existing structures or mechanisms rather than creating new ones (Jacob, 1977). Indeed, a growing body of evidence points to a substantial and, until recently, unrecognized continuity in anatomy, biochemistry, physiology and genetics between the bryophyte and polysporangiophyte grade (Cooke *et al.*, 2004; Raven and Edwards, 2004; Floyd and Bowman, 2007; Ligrone *et al.*, 2012). We infer that the sporophyte shoot probably evolved by modification of an ancestral bryophytic pattern of embryo development. The present paper explores the origin of the sporophyte shoot in the putative bryophytic ancestor of polysporangiophytes and its possible evolutionary relationship with the sporophyte in mosses and hornworts.

STOMATA AS A GUIDELINE FOR ANALYSING THE ORIGIN OF THE SPOROPHYTE SHOOT

Stomata are one of the most distinctive features of the sporophyte shoot. By adjusting their aperture in response to multiple external and internal signals, stomata work to maintain a favourable balance of water loss and CO_2 uptake (Brodribb *et al.*, 2009; Lawson, 2009; Brodribb and McAdam, 2011). Stomata also occur in the sporophyte of mosses and hornworts (Paton and Pearce, 1957), and molecular evidence (Bergmann and Sack, 2007; Peterson *et al.*, 2010; Rychel and Peterson, 2010; Chater *et al.*, 2011) supports a monophyletic origin of stomata in the putative common ancestor of the three lineages. Because of this, mosses, hornworts and polysporangiophytes have been collectively referred to as the stomatophytes (Ligrone *et al.*, 2012).

As an adaptation pivotal to the evolution of homeohydry and of large-sized land plants (reviewed in Raven, 2002; Proctor, 2007), stomata have had a tremendous impact on the biology and geochemistry of the planet (for reviews, see Beerling, 2007; Berry *et al.*, 2010). However, the existence of stomata in poikilohydric bryophytes indicates that these structures might not have been associated with homeohydry since their origin. It has been suggested that stomata evolved in the sporophyte of the ancestral stomatophyte as a means to divert water and solutes from the parental gametophyte and, at maturity, also facilitate spore dispersal (Ligrone *et al.*, 2012).

The ancestral stomata were most probably simple apertures lacking an opening/closing mechanism, and arguably more advanced functions evolved in parallel with an increasingly complex sporophyte body. Unfortunately, the current understanding of the evolution of stomatal responsiveness is incomplete, due in part to conflicting data in bryophytes and early diverging tracheophytes (Brodribb and McAdam, 2011; Chater *et al.*, 2011; Ruszala *et al.*, 2011; McAdam and Brodribb, 2012).

More informative in the context of the present analysis is stomatal distribution. In mosses, stomata are localized in the sporangium, and the same has been assumed for the putative ancestral stomatophytes; stomata are expressed throughout the fertile sporophyte axis in hornworts, whereas in polysporangiophytes they are found in the sporophyte shoot (Ligrone *et al.*, 2012). Stomata also occurred in the sporangia of Early Devonian polysporangiophytes such as *Aglaophyton major*, *Nothia aphylla* and *Cooksonia pertoni* (Edwards *et al.*, 1998); hence, the lack of stomata in the sporangia of extant polysporangiophytes is probably a loss (Ligrone *et al.*, 2012). We conclude that (1) the ancestral stomatous part of the stomatophyte sporophyte is the sporangium; and (2) stomatal distribution is a phylogenetic trait holding essential clues on the origin of the sporophyte shoot and its relationship with the sporophyte in mosses and hornworts.

EVOLUTION OF SPOROPHYTE BODY PLANS IN STOMATOPHYTES AND ORIGIN OF THE SPOROPHYTE SHOOT

In a previous analysis of land plant evolution, we argued that the sporophyte of ancestral stomatophytes had a vascularized

seta and a sporangium with stomata, air spaces and chlorenchyma (Ligrone *et al.*, 2012). This pattern of sporophyte anatomy was assumed to be plesiomorphic in mosses, and the lack of one or more of the above characters in early diverging or advanced moss lineages was considered to be the result of independent loss (Ligrone *et al.*, 2012). In line with the above considerations, here we assume that the sporophyte of the ancestral stomatophyte developed from a moss-like spindle-shaped embryo (Cooke *et al.*, 2004; Sakakibara *et al.*, 2008; Uzawa and Higuchi, 2010; Ligrone *et al.*, 2012) consisting of a sporangium primordium, a haustorium of hypobasal derivation and, between these, a unifacial basal meristem (Fig. 1A, B).

A major character distinguishing the hornworts and polysporangiophytes from mosses is an amplification of the part of the sporophyte body expressing stomata and photosynthetic tissue. This coincides with the whole sporangium in the hornworts and the sporophyte shoot in polysporangiophytes.

The early embryo of hornworts consists of two tiers of four cells, with eight cells in total. This octant stage is sharply different from the spindle-shaped embryo of mosses but is found in most pteridophyte embryos during initial development (Johnson and Renzaglia, 2008, 2009). The upper and lower tier of the 8-celled hornwort embryo are a sporangium primordium and a foot primordium, respectively (Renzaglia, 1978). As in mosses, a meristematic area develops at the base of the sporangium primordium after delineation of an endothecium and amphithecium (regions restricted to the sporangium of mosses and hornworts) but before the sporogenous tissue (archesporium) is defined (Renzaglia, 1978; Fig. 1C). As in mosses, this meristem is unifacial and produces new tissue acropetally; hence, it is referred to as the basal meristem. Unlike mosses, however, the basal meristem in hornworts reproduces the developmental pattern of the sporangium primordium and remains active for the life of the sporophyte. Consequently, the sporangial axis of the hornwort sporophyte is an indeterminate structure that arises almost entirely from the basal meristem, with a very minor contribution from the sporangium primordium (Fig. 1D).

With reference to the putative moss-like ancestral embryo, we suggest that the hornwort sporophyte developmental pattern evolved through ectopic expression of the morphogenetic programme of the sporangium in the meristematic area ancestrally deputed to producing the seta. Having lost the tissue contribution from the basal meristem, the foot acquired a bulbous shape and the seta was suppressed (Fig. 1D).

The key event marking the divergence of the polysporangiophyte lineage was the evolution of a SAM, a bifunctional apical meristem performing two alternative morphogenetic programmes. In the vegetative mode, the SAM produces an indeterminate sterile axis, i.e. the sporophyte shoot (Fig. 1F), and in the reproductive mode a determinate sporangial axis (Fig. 1G), the transition from vegetative to reproductive growth involving the loss of the initial cell(s) and consequent end of indeterminate growth (reviewed by Stahl and Simon, 2010). An apical meristem either evolved *de novo* from the sporangium primordium or, more simply, the meristematic area shifted from an intercalary to a superficial location. Based on the common octant stage of present-day hornwort and polysporangiophyte embryos, we speculate that the latter is the likely scenario and that this transition, involving the loss of the ancestral sporangium primordium, took place after the basal meristem had acquired an indeterminate character and had been co-opted into the production of sporangial tissue. The conversion of the newly evolved apical meristem into an ancestral SAM entailed two further key changes. One was the interpolation of a phase of vegetative growth preceding the development of the archesporium; the other an inversion in the polarity of formative cell divisions. These were no longer integrated between already determined tissues as in hornworts but rather were positioned in such a way that the initial cell(s) could maintain an apical position and thus 'lead the way' for iterative growth. We postulate that, although lacking the ability to produce leaf primordia, the apical meristem of the leafless unbranched sporophyte in ancestral polysporangiophytes possessed the fundamental properties of a SAM, i.e. an indeterminate meristematic activity and delayed sporogenesis (Fig. 1E).

DISCUSSION AND CONCLUSIONS

Appearing ancestrally in a poikilohydric sporophyte and most probably originally lacking a functional link with photosynthesis (Ligrone *et al.*, 2012), stomata have turned out to be a key tool affording a better control of water balance and more efficient photosynthetic activity. If terrestrialization gave plants access to greater relative amounts of carbon dioxide, the evolution of stomata and air spaces was the innovation that enabled them to exploit this opportunity to its full extent (Raven, 1996, 2002; Raven and Edwards, 2004). Arguably, the conversion of ancestral stomata into the multisensing 'smart' valves of present-day angiosperms was gradual (Franks and Farquhar, 2007; Brodribb *et al.*, 2009). Recent studies suggest that the stomata in hornworts (J. G. Duckett, Natural History Museum, London, UK, unpubl. res.), lycophytes and ferns (Brodribb and McAdam, 2011; McAdam and Brodribb, 2012) lack key responses to abscisic acid; moreover, hornwort stomata exhibit only partial closure under water stress and remain open when dead (J. G. Duckett, Natural History Museum, London, UK, unpubl. res.). It is nevertheless significant that in mosses and hornworts stomata are associated with well-defined chlorenchyma with schizogenous air spaces (Raven, 1996; J. G. Duckett, Natural History Museum, London, UK, unpubl. res.) and, hence, most probably the anatomical organization was in place in the ancestral stomatophytes to support the evolution of opening and closing cycles.

Once they appeared, stomata were the major driver of sporophyte evolution. We suggest that the ancestral role of the basal meristem in stomatophytes was to push the expanding sporangium out of gametophytic tissues, thereby permitting the stomata and underlying air spaces to become functional in gas exchange as early as possible. This is the condition typically observed in extant mosses (Uzawa and Higuchi, 2010; Ligrone *et al.*, 2012). The conversion of the basal meristem into an indeterminate meristem producing sporangial tissue instead of a seta entailed a significant amplification of the stomata–air space–chlorenchyma system in ancestral

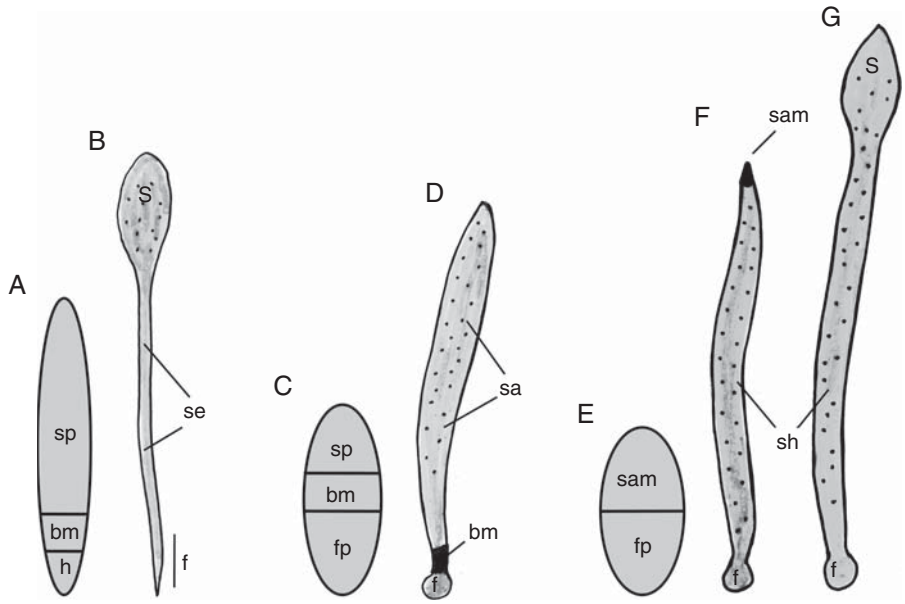

FIG. 1. Embryo and mature sporophyte in stomatophytes. (A, B) A putative embryo and sporophyte in ancestral stomatophytes; the embryo is assumed to consist, as in present-day mosses, of three organogenetic areas: a sporangium primordium (sp), a basal meristem (bm) and a haustorium (h); these generated, respectively, a sporangium (s), a seta (se) plus the upper part of the foot (f), and the lower part of the foot in the mature sporophyte. (C, D) An embryo and mature sporophyte in hornworts. (C) An embryo consisting of a sporangium primordium (sp), a basal meristem and a foot primordium (fp). (D) The basal meristem (bm) is active for sporophyte life and produces an indeterminate sporangial axis (sa). (E–G) A putative embryo, immature and mature sporophyte in an ancestral polysporangiophyte. The embryo is assumed to consist of an ancestral shoot apical meristem (sam) and a foot primordium (fp). Post-embryonic growth from the ancestral SAM produced a leafless, uniaxial indeterminate sporophyte shoot (sh) and eventually a terminal sporangium (s). f, sporophyte foot. Stippling indicates stomata. The embryo stages are not to scale with the other stages.

hornworts and polysporangiophytes. It is parsimonious to assume that this condition evolved before the two lineages diverged. Possibly, a pre-adaptation leading the way to the loss of the seta and diversion of the basal meristem towards the production of sporangial tissue was a reduction of mechanical constriction from the gametophytic tissue surrounding the young sporophyte. The resulting sporophyte body plan remained substantially unaltered in the hornwort lineage, except in the genus *Notothylas* which has a reduced sporophyte (Renzaglia *et al.*, 2009). The longitudinal division of the zygote is a hornwort apomorphy possibly related to the sunken nature of the archegonium (Renzaglia *et al.*, 2009). The same innovation appeared independently in eusporangiate ferns (Johnson and Renzaglia, 2009).

In the scenario proposed here, further elaboration of the sporophyte body plan and underpinning embryo organization in the polysporangiophyte lineage involved suppression of the sporangium primordium, meristem apicalization and a temporal splitting of the developmental programmes for the vegetative (epidermis with stomata and photosynthetic tissue) and reproductive part of the sporangium (archesporium and associated tissues). The key agent of positional/temporal shifting and silencing of developmental programmes is homeotic mutation, a class of mutations affecting regulatory genes and/or their targets, and recognized as a major driver of body plan change in plants and animals (Vinicius, 2010; Pires and Dolan, 2012). In a broad sense, homeotic genes also embrace genes encoding small RNAs associated with the timing of developmental transitions (Moss, 2007; Poethig, 2009).

The hypothesis that the ancestral SAM arose from an embryo area pre-determined to produce sporangial tissue implies interpretation of the sporophyte shoot as a sterilized sporangial axis intercalated between the early embryo and the fertile sporangium. Sterilization and diverted development of reproductive structures is a recurrent mechanism of morphological innovation in plants. Striking examples are the modification of marginal flowers in the inflorescences of Asteraceae into sterile structures for attraction of pollinators, the conversion of stamens into staminodes and even the origin of petals from parts of the androecium (reviewed by Crane and Kenrick, 1997). Other likely examples are microphylls in lycophytes and interseminal scales in Bennettitales, both suggested to be derived from sterilized sporangia, for the former as an alternative to the enation model (Crane and Kenrick, 1997). Indeed, partial sterilization of the sporangium is also the likely mechanism at the origin of the capsule neck or apophysis in peristomate mosses, that is a specialized sporangium segment containing stomata and chlorenchyma but lacking archesporial tissue (Goffinet *et al.*, 2009). It may also be observed that sterilization of potential sporogenous cells is at the very origin of the sporophyte vegetative tissue in embryophytes (Hemsley, 1994).

With reference to the putatively ancestral condition found in extant mosses, the sporophyte body plans in hornworts and polysporangiophytes may be viewed as the expression of opposite heterochronic events, i.e. an anticipation (progenesis) and a delay (neoteny), respectively, in the development of the sporangium (Gould, 1977; Alberch *et al.*, 1979; Ridley,

2004). In hornworts, the sporophyte starts producing archesporial tissue in the embryo and young sporophyte (Renzaglia *et al.*, 2009), whereas in polysporangiophytes the sporophyte vegetative body grows for a relatively long time before sporangial development initiates. In either case, the result is a pedomorphic sporophyte permanently retaining juvenile characters: an active meristem and the potential to produce spores.

The evolutionary model presented here assumes that a moss-like spindle-shaped embryo is plesiomorphic in stomatophytes and that the embryo and sporophyte body plans in hornworts and polysporangiophytes arose by sequential elaboration of this ancestral pattern. This model is consistent with molecular phylogenies as well as with substantial similarity in the sporophyte body plan of mosses and liverworts, the latter being the earliest diverging extant embryophyte lineage (Qiu *et al.*, 2006, 2007; Qiu, 2008; Chang and Graham, 2011). An alternative scenario assuming the ancestral stomatophyte embryo to be similar to the globular embryo of hornworts and the spindle-shaped embryo to be an apomorphy of mosses would be less consistent with phylogenetic evidence but would still be compatible with our model, its main implication being, in our opinion, that the seta of mosses should be interpreted as an innovation derived from a sterilized sporangial segment. An independent origin of the moss seta by partial sterilization of the sporangial axis appears to be a plausible hypothesis, although less parsimonious than our model because it implies two separate events of sporangial sterilization in stomatophytes and rules out homology with the liverwort seta. A polysporangiophyte-like plesiomorphic embryo would not only be at sharp variance with phylogeny but would also imply a sequence of events far less parsimonious than the scenarios outlined above.

Unlike determinate sporophyte development in liverworts and mosses, sporophyte development in hornworts and polysporangiophytes is essentially a stochastic process involving an unpredictable number of cell divisions although producing highly regular forms. Arguably, the evolution of an indeterminate body increased the photosynthetic and reproductive potential of the sporophyte but possibly also amplified a conflict of interest with the parental gametophyte, at least in terms of allocation of water and mineral nutrients (Haig and Wilczek, 2006). Probably this was the major factor driving sporophyte evolution towards autonomy in polysporangiophytes. Because of the absence of vascular tissue, stomatal transpiration in the hornwort sporophyte presumably is lower than might be expected in a comparable vascularized system, and this may have worked in reducing potential conflict with the gametophyte; moreover, the basal position of the meristem in the hornwort sporophyte is probably incompatible with branching, which presumably was an essential prerequisite for evolving root-like structures and gaining autonomy (Ligrone *et al.*, 2012). Taken together, these two factors may account for the retention of a bryophytic life cycle in hornworts.

The root apical meristem (RAM) and leaf primordium present in the embryo of extant polysporangiophytes (Johnson and Renzaglia, 2008, 2009) are additions that followed the evolution of branching and were associated with roots and leaves; each appeared at least twice independently, in lycophytes and euphyllophytes (Raven and Edwards, 2001; Pires and Dolan, 2012). The leaf primordium most probably resulted from further segregation from the SAM (Johnson and Renzaglia, 2009), whereas the origin of the root is more uncertain (Raven and Edwards, 2001; Pires and Dolan, 2012). Several regulatory genes that in angiosperms control SAM functioning have homologues expressed in the RAM (Stahl and Simon, 2010), suggesting that the RAM originated by duplication of the SAM.

In the last two decades, molecular research has identified several classes of genes involved in the control of the SAM. Major examples include Class III HD-Zip and Class 1 KNOX transcription factors, both essential for the initiation and maintenance of the SAM in angiosperms, gymnosperms and ferns, and *KANADI* genes whose ectopic expression in the SAM causes terminal differentiation in arabidopsis (Emery *et al.*, 2003; Floyd *et al.*, 2006; reviewed by Floyd and Bowman, 2007). The functioning of the SAM in arabidopsis and other angiosperms also depends on a regulatory loop between *clavata* and *wuschel* genes (Schoof *et al.*, 2000; Taguchi-Shiobara et al., 2001; Suzaki et al., 2006). MIKC MADS-box genes control flower development and include the *ABC* homeotic genes (Soltis *et al.*, 2007). The *LEAFY* gene is involved in the control of the transition from vegetative to reproductive growth in angiosperms by regulating the transcription of *ABC* genes; AP2 genes, a gene family associated with floral development in angiosperms, include sequences involved in stem cell maintenance and transition from vegetative to reproductive growth in both early diverging and more derived tracheophytes (reviewed by Floyd and Bowman, 2007).

The recent addition of the near complete genome sequence of the moss *Physcomitrella patens* (Rensing *et al.*, 2008) has permitted the identification of moss homologues for most of the above-mentioned genes (Tanahashi *et al.*, 2005; Singer and Ashton, 2007; Sakakibara *et al.*, 2008; see also reviews by Floyd and Bowman, 2007; Shaw *et al.*, 2011; Pires and Dolan, 2012).

The overall evidence indicates that the evolution of the complex gene networks underpinning sporophyte development in angiosperms entailed repeated events of duplication, functional co-option and neo-functionalization of genes already present in the ancestral genotype. A similar picture is produced by comparative analysis of growth-promoting hormones in early diverging and more derived land plants (Ross and Reid, 2010). So, it is increasingly evident that understanding the molecular bases of sporophyte evolution and development requires filling the knowledge gap between angiosperms and bryophytes. Essential to this purpose is nuclear genome sequencing in liverworts and hornworts.

It is anticipated that the present analysis will inspire future research and provide a framework for data testing and interpretation. Even within the boundaries of limited current knowledge, the hypotheses presented are amenable to experimental testing. Research on shared genetic signatures of sporophyte meristems in mosses, hornworts and polysporangiophytes not only might permit assessment of homology but also is a promising line of enquiry for genetic markers of the transition from embryonic to post-embryonic development. The same applies for testing the hypothesis of a common

origin of indeterminate sporophyte development in hornworts and polysporangiophytes. Likely candidates include homologues of Class 1 Knox and Class III HD-Zip genes. In *Physcomitrella* the former control formative cell division in the sporangium primordium and basal meristem whereas they are not expressed in the gametophyte (Sakakibara *et al.*, 2008). Class III HD-Zip homologues are expressed in the haploid bodies of *Chara*, the three bryophytic lineages, and the fern *Ceratopteris* (Floyd *et al.*, 2006), and expression has also been reported in the sporophyte in the hornwort *Phaeoceros* and several tracheophytes including *Ceratopteris* (Floyd *et al.*, 2006). Our model points to differences in the timing of spore production as a major character distinguishing mosses, hornworts and polysporangiophytes. Expression and functional analysis of genes controlling the transition from vegetative to reproductive growth, notably EP2 genes (Floyd and Bowman, 2007), is likely to produce data useful to test our model and, in particular, our heterochronic interpretation of sporophyte evolution in hornworts and polysporangiophytes.

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