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Review

Morphological evolution in land plants: new designs with old genes

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The colonization and radiation of multicellular plants on land that started over 470 Ma was one of the defining events in the history of this planet. For the first time, large amounts of primary productivity occurred on the continental surface, paving the way for the evolution of complex terrestrial ecosystems and altering global biogeochemical cycles; increased weathering of continental silicates and organic carbon burial resulted in a 90 per cent reduction in atmospheric carbon dioxide levels. The evolution of plants on land was itself characterized by a series of radical transformations of their body plans that included the formation of three-dimensional tissues, *de novo* evolution of a multicellular diploid sporophyte generation, evolution of multicellular meristems, and the development of specialized tissues and organ systems such as vasculature, roots, leaves, seeds and flowers. In this review, we discuss the evolution of the genes and developmental mechanisms that drove the explosion of plant morphologies on land. Recent studies indicate that many of the gene families which control development in extant plants were already present in the earliest land plants. This suggests that the evolution of novel morphologies was to a large degree driven by the reassembly and reuse of pre-existing genetic mechanisms.

Keywords: evolution; development; root; leaves; flowers; regulatory genes

1. THE HISTORY OF LAND PLANTS

(a) *Overview of land plant evolution*

Land plants (embryophytes) evolved from freshwater multicellular algae, probably related to the extant charophyte groups Charales or Coleochaetales [1–4]. Together, land plants and charophytes form a monophyletic group, the streptophytes, which is sister to the other green algae: the chlorophytes (figure 1). The most basal and simple streptophytes, such as *Mesostigma*, are unicellular, but a progressive transition towards complex multicellularity occurred during the evolution of the different groups of streptophytes. Charophytes evolved many features that are plesiomorphic for land plants, such as hexameric cellulose synthases, a phragmoplast, plasmodesmata, apical growth and a placenta [1,12]. However, it was the transition of streptophytes to terrestrial environments that was associated with the evolution of the key features that define land plants, such as a multicellular sporophyte, retention of the zygote and embryo within the female gametophyte, and apical cells with three cutting faces that allow the generation of three-dimensional parenchymatous tissues [12,13].

The oldest fossil evidence for plants on land comes from spores and tissue fragments extending back through the Mid-Ordovician, 470 Ma [14–16]. The morphology of these microfossils suggests an affinity with extant liverworts, although the first macrofossils of liverworts appear only in the Middle Devonian, around 390 Ma [17]. The first land plant macrofossils, represented by the sporophytes of *Cooksonia* and similar forms, appear on older Mid–Late Silurian strata, around 425 Ma [14,18]. It is generally suggested that the absence of preserved gametophytes in the fossil record at this time, 425 Ma, results from their low preservation potential—the remains decomposed before they could be fossilized. Nevertheless, fossils from the Early Devonian Rhynie Chert indicate that the gametophytes of early land plants were complex (including stomata and conducting elements) and often resembling the gametophytes of extant liverworts [19].

The oldest evidence for the existence of vascular plants comes from trilete spores found in Upper Ordovician sediments, over 443 Ma [20], although tracheid fossils can only be identified in Late Silurian strata, over 415 Ma [14]. Vascular plants went on to become the dominant vegetation on terrestrial environments, while liverworts, mosses and hornworts are the sole descendants of the first, non-vascular, plants. By the Late Silurian (around 425 Ma), the now extinct rhyniophytes, zosterophylls and the first lycophytes had evolved [19,21]. The Devonian period (415–360 Ma) was characterized by an explosion in the diversity of land plants [21], caused by a radiation of vascular plants with a dominant sporophyte generation that colonized drier habitats and were no longer restricted to damper areas [22]. The advantages of an increased dominance of the sporophyte in land plants were probably owing to the potential for the production and air-dispersal of numerous spores after a singular

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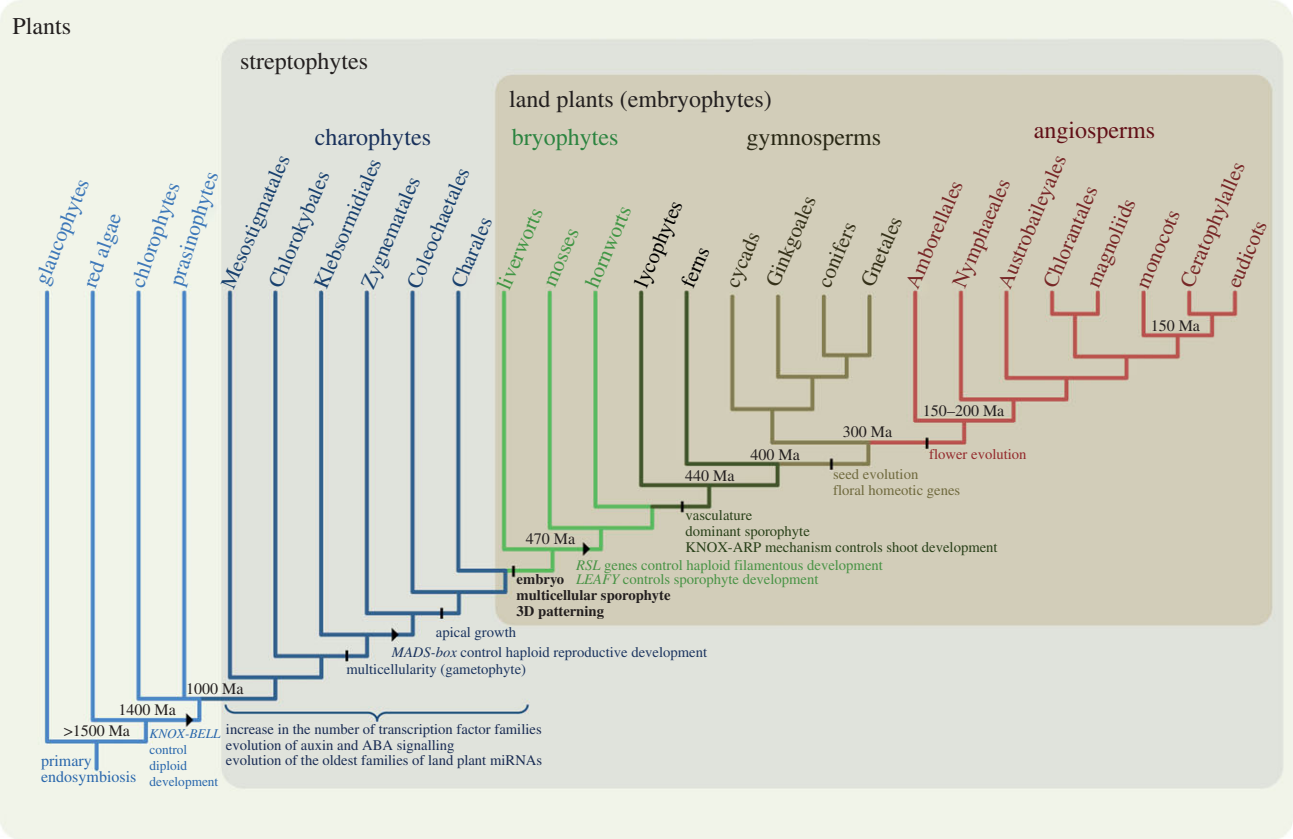

Figure 1. Phylogenetic relationships between the major groups of extant plants. Key events that occurred during plant evolution are indicated; in cases where enough functional data are not available, the minimum origin is indicated by an arrowhead. The estimated divergence times are indicated in millions of years ago (Ma). The phylogenetic relationships between different plant groups are based on earlier studies [3–7]. The estimated divergence times are based on previous studies [8–11]. See also the main text for more details.

water-dependent fertilization event. Many important extant groups, including horsetails, ferns (which together constitute the monilophytes) and the first seed plants, appeared and diversified during this period [21].

By the Late Carboniferous period, around 300 Ma, much of the land surface was covered by large forests of pteridosperms (seed ferns), lycophytes, tree ferns and sphenopsids [22–25]. Gymnosperms appeared during this period [19] and became dominant in the world flora between the Permian and the Late Cretaceous period (between 260 and 70 Ma) [25]. Basal angiosperms, magnoliids, early monocots and early eudicots appeared almost simultaneously during the Early Cretaceous (100–145 Ma) [19,26] and later radiated and became dominant in a majority of habitats from the Late Cretaceous (100–65 Ma) until the present day [25].

(b) *Impact of the land plant radiation on the carbon cycle*

The radiation of land plants during the Devonian period caused large changes to the global carbon cycle resulting in a decrease in atmospheric carbon dioxide (CO_2) levels. This reduction was brought about by at least two different processes: enhanced chemical weathering of silicate minerals and increased amounts of carbon burial. During the weathering reaction, CO_2 from the atmosphere dissolves in ground water forming carbonic acid, which reacts with silicates releasing bicarbonate that moves to the ocean through rivers and streams. Once in the ocean, bicarbonate reacts with calcium or magnesium ions, forms carbonate precipitates and accumulate in sediments. Silicate weathering is thereby a mechanism by which CO_2 is removed from the atmosphere and buried as carbonate-rich sediments for millions of years. Plants enhance rates of silicate weathering in at least two ways. Firstly, the roots of plants physically break up rocks, increasing the surface area of the exposed rock to carbonic acid. Secondly, plants and their mycorrhizal symbionts secrete organic acids that break down silicate minerals. Before deep-rooting plants evolved, silicate weathering was restricted to the surface layers because early diverging groups of land plants were 'rooted' in place by a system of hair-like rhizoids and horizontal stems that penetrated the top few centimetres of the soil. Thus, the evolution of the first large plants with deep-rooting systems in the Devonian caused a step change increase in global silicate weathering [27]. The consequent increase in CO_2 drawdown was the major factor that decreased atmospheric CO_2 levels in the Mid-Palaeozoic [27–29].

The high rates of primary productivity by these Devonian plants led to the burial of large amounts of carbon on the continents for the first time, resulting in the formation of new terrestrial carbon pools [28,30]. There were a variety of sources of plant-derived carbon for burial. These included (i) plant-derived particulate

organic matter (detritus); (ii) carbon secreted into the soil in the form of organic molecules such as citric acid intermediates; (iii) carbon-rich molecules that were transferred to mycorrhizal symbionts. This large-scale production of carbon on the continental surfaces led to the development of the first complex soils and extensive peat (undecomposed plant-derived detritus) deposits on flooded regions of the continental shelf. Today, there is 2300 Gt in the soil carbon pool—including carbon in soils and peat—which is 3.3 times the amount present in the atmosphere. This formation of a new carbon pool demonstrates the impact of the radiation of the land plants on the global carbon cycle [31].

These early land plants also depended on the rock-derived minerals as a source of essential inorganic nutrients such as potassium and phosphorus. The spread of terrestrial vegetation and the evolution of large plants with complex root systems is therefore likely to have led to an increase in the flux of phosphorus to the ocean, which in turn will have promoted marine productivity and further increased the drawdown of carbon from the atmosphere and accumulation in carbonate ocean sediments [28,30,32]. Together, these events caused a dramatic decrease in atmospheric CO_2 levels and an increase in the levels of O_2 which are considered to have contributed, at least in part, to the global cooling that occurred during the Early Carboniferous.

While the evolution and radiation of land plants impacted on the Palaeozoic carbon cycle, these changes in turn fed back on plant evolution. There is evidence that changes in the carbon cycle may have been a driving force behind the evolution of plant form; falling CO_2 levels during the Devonian period probably had an impact on the evolution of leaf size [33]. The heat produced by the absorption of solar radiation of leaves is conducted away through evaporative cooling through stomata, and stomatal densities are inversely related to atmospheric CO_2 levels. It has been hypothesized that large leaves could not have evolved until CO_2 levels fell below a critical level, when stomatal density would have been sufficient to provide cooling. In the absence of such cooling mechanisms, leaves would overheat. This means that high levels of CO_2 at the beginning and middle of the Devonian period would have constrained leaf size. Then as CO_2 levels decreased in the Late Devonian and Carboniferous periods, stomatal density increased and thereby enhanced the cooling capacity of leaves. This increased cooling capacity would have removed the growth constraint imposed by overheating and leaves increased in size. This is an example of how feedbacks between the carbon cycle and plants constrained plant form and impacted on morphological evolution.

2. GENES AND THE EVOLUTION OF PLANT DEVELOPMENT

(a) *Sporophyte and gametophyte*

A key characteristic of land plants is that their life cycle is composed of two distinct multicellular generations: a haploid gametophyte and a diploid sporophyte. By contrast, only the zygote cell is diploid in streptophyte algae such as *Chara* and *Coleochaete*. An alternation of two multicellular generations has evolved several times in different groups of algae [34] but it appears to have evolved only once in the streptophytes. Two major theories have historically addressed the origin of the alternation of generations in land plants: the homologous (or transformation) and the antithetic (or interpolation) theories (reviewed by Blackwell [35]). The homologous theory proposed that land plant ancestors had an alternation of isomorphic generations; this theory has currently little support, except for the existence of early Devonian fossils with almost isomorphic generations [21]. By contrast, the more widely accepted antithetic theory suggests that the sporophyte originated through the intercalation of mitotic divisions in the zygote before meiosis, resulting in a diploid embryo being retained on a gametophytic thallus [36]; the sporophyte would then gradually evolve from a parasitic dependence on the gametophyte into a dominant, physiologically independent organism. The antithetic theory is supported by the dominance of gametophytes over sporophytes in bryophytes and by the absence of sporophytes in charophyte algae.

One prediction that can be made from the antithetic theory is that the evolution of the sporophyte involved the recruitment of ancient genes and regulatory networks from the pre-existing gametophyte generation; these networks would then diversify and promote morphological diversification in the sporophyte generation. Support for the hypothesis that genes and regulatory networks were recruited from the gametophyte to the sporophyte comes from transcriptomic studies. These showed that the differences in gene expression between gametophyte and sporophyte are greater in angiosperms than in mosses; moreover, many homologues of moss gametophyte-biased transcription factors are preferentially expressed in the sporophyte of angiosperms [37].

The recruitment of genes that controlled gametophyte-specific activities in ancestral plants to regulatory roles in the sporophyte generation of relatively derived plants is exemplified by the evolution of type II MADS-box transcription factors. A single type II MADS-box gene functions during haploid reproductive cell differentiation in different charophyte algae [38]. Type II MADS-box genes radiated in land plants and formed two groups: the MIKC^c and MIKC* [39]. MIKC* genes have retained a gametophyte function in bryophytes [40] and angiosperms [41]; conversely, MIKC^c genes are expressed in both the gametophyte and sporophyte tissues in mosses and ferns [42–44], but are mostly restricted to the sporophyte in *Arabidopsis*, where they function as the most important floral homeotic genes [41]. This suggests that the MIKC^c genes progressively acquired roles in sporophyte development during the evolution of vascular plants. Another example was the discovery that *RSL* genes control the differentiation of root hairs in the angiosperm *Arabidopsis thaliana*, and rhizoid and caulonema cells in the moss *Physcomitrella patens* [45]. Root hairs, rhizoids and caulonemata are structures that fulfil similar rooting functions, but whereas root hairs are tubular projections from epidermal cells of the root in the sporophytic life cycle stage, rhizoids and caulonemata are multicellular filamentous structures that grow in the gametophytic life cycle stage. This suggests that *RSL* class I genes that controlled the development of rooting filaments in the gametophyte

of early land plants were later recruited to control the development of root hairs in the sporophyte generation [45]. These examples suggest that the elaboration of the sporophyte generation, and particularly the large radiation of morphological forms during the Devonian period, was partially achieved through the recruitment of genes and genetic mechanisms that had previously evolved and functioned in the gametophyte generation of charophytes and early land plants.

Some genes also have ancient functions that are restricted to the diploid phase of the life cycle (zygote and/or sporophyte). In the unicellular chlorophyte *Chlamydomonas reinhardtii*, the formation of a heterodimeric complex with the proteins Gsm1 and Gsp1 is sufficient to initiate the diploid phase of the life cycle [46]; Gsm1 and Gsp1 are members of the TALE superclass of homeobox proteins, which in land plants include the KNOX and BEL classes [47]; KNOX and BEL proteins are important regulators of sporophyte development in mosses and vascular plants [48–50], suggesting that the function of TALE proteins is restricted to the diploid phase of the life cycle in both chlorophyte algae and in land plants. Similarly, the floral meristem regulator LEAFY also functions specifically in the sporophytes of mosses, ferns and angiosperms [51,52]. These examples suggest that some genes have ancient functions that are exclusive to either the sporophyte or the gametophyte generation.

The cues that mediate the transition of the different stages of the life cycle are likely to involve epigenetic mechanisms. Okano *et al.* [53] and Mosquna *et al.* [54] showed that *PpCLF* and *PpFIE*, moss genes encoding putative subunits of a Polycomb group complex that regulates epigenetic states through chromatin modification, are required for the correct establishment of sporophyte and gametophyte identity: loss-of-function *Ppclf* and *Ppfie* mutants develop sporophyte-like bodies in the place of gametophores. The phenomenon of apogamy (development of sporophytes from gametophytes without fertilization) is long known to occur in fern and bryophyte species [55], but the discoveries of Okano *et al.* [53] and Mosquna *et al.* [54] provide a glimpse into the molecular mechanisms that provide the epigenetic context of life-cycle transitions.

(b) *Leaf evolution*

Leaves have evolved multiple times in land plants. There are two types of leaf-like structures in vascular plants, microphylls and megaphylls. Microphylls, small and simple leaves with a single unbranched vein, are hypothesized to have evolved during the Silurian/Early Devonian [19] through either the vascularization of stem enations, the reduction of flattened lateral branches or the sterilization of sporangia [56]. The first leaves of euphyllophytes (megaphylls), which have a complex venation pattern, are likely to have evolved multiple times by the Late Devonian/Carboniferous, probably through the planation (flattening) and webbing of branch systems [19]. The megaphylls of seed plants and ferns are probably not homologous, but determining the total number of independent origins of leaves in euphyllophytes depends on the future resolution of a complex phylogeny of Devonian and Carboniferous fossils [57,58]. Mosses and liverworts also have leaf-like structures, but these are not homologous to either microphylls or megaphylls.

The formation of leaves involves a transition from indeterminate growth in shoot apical meristems into a determinate growth programme. The indeterminacy of the shoot apical meristem is maintained by KNOX transcription factors; in different angiosperms, the initiation of leaf determinate growth requires that KNOX genes are negatively regulated by ARP proteins in leaf primordia. A similar KNOX–ARP mechanism operates during microphyll development in lycophytes, despite the independent origin of microphylls and megaphylls [59]. This suggests that the mechanisms for regulating determinacy and indeterminacy were present in the common ancestor of vascular plants and were recruited independently to control leaf initiation. By contrast, a mechanism involving Class III HD-Zip transcription factors that pattern stem vasculature was co-opted for the adaxial/abaxial patterning of leaves in seed plants but not in lycophytes [60], reflecting the independent origin of microphylls and seed plant megaphylls.

In angiosperms with entire (unlobed) leaves, KNOX expression is restricted to the shoot apical meristem and disappears from the cells of P0—the site where the next leaf primordium will form. This change in KNOX expression signals the transition between indeterminate and determinate growth. KNOX genes then remain transcriptionally inactive for the remainder of the development of entire leaves. By contrast, in the lobed leaves of some seed plants and of fern fronds, KNOX genes are transcriptionally reactivated sometime after P0 [59,61–63]. Given that lobed leaves evolved independently a number of times, this means that KNOX gene expression in post-P0 primordia was activated each time lobed leaves evolved. Furthermore, the evolution of entire leaves from lobed leaves in the *Brassicaceae* genus was accompanied by the loss of KNOX expression in leaves [64]. Thus, the reactivation of KNOX expression during compound leaf development appears to have had multiple evolutionary origins [62] and provides a remarkable example of how small changes in the spatial expression of a transcription factor can cause the evolution of a multitude of different morphologies.

(c) *Root evolution*

The successful colonization of terrestrial environments required the evolution of multicellular organs that actively penetrate the substrate, anchor the plant and retrieve the mineral nutrients necessary for plant growth. The rooting function in free-living gametophytes of non-vascular plants (and to some extent in a few aquatic charophytes and chlorophyte algae) is performed by a system of rhizoid filaments. True roots comprising a specialized axis, a root cap, an endodermis and an endogenous origin of lateral branches are found only in the sporophytes of vascular plants [65]. The earliest vascular plants did not have specialized root axes, but the lycophyte clade had evolved roots by the Early Devonian; on the other hand, there is no evidence of roots in other vascular

plants until the middle Devonian [65,66]. This suggests that roots evolved at least twice in land plants and that the occurrence of an endodermis, endogenous branching, and an endodermis in the roots of both lycophytes and euphyllophytes can be interpreted as the result of convergent evolution. Within the euphyllophytes, a fundamental difference in the anatomy of embryonic roots suggests that roots evolved independently in seed plants and in free-sporing monilophytes [65]. As discussed above, the development of root hairs in the root of angiosperms and of rhizoids in the gametophyte of mosses is controlled by RSL class I proteins [45]. Given the independent origin of roots in vascular plants, it will be interesting to determine if the development of root hairs/rhizoids in lycophytes and monilophytes also involved the recruitment of *RSL* class I genes. Many questions remain regarding the exact homology of shoots and roots: in angiosperms, several regulatory factors that control shoot apical meristem (such as WUS and CLV3) have homologues that regulate the function of the root apical meristem (reviewed in [67]). It will be interesting to determine if these regulators are also required for the development of the independently derived root meristem of monilophytes and lycophytes.

(d) *Flower evolution*

The evolution of seeds and flowers were major events in land plant evolution and probably the most important factors responsible for the dominance of gymnosperms and angiosperms on land floras for the past 250 Myr. Little is known regarding the genetic mechanisms that guided the evolution of seeds in the Middle Devonian, but the evolution of flowers has received considerable attention by developmental and evolutionary biologists.

The earliest fossils of flowers are from the Early Cretaceous (around 125 Ma), and indicate that a rapid diversification of floral forms occurred very early in angiosperm evolution [26]. The evolution of flowers in angiosperms involved the transformation of unisexual gymnosperm reproductive structures into a hermaphrodite structure. Different theories providing an explanation for this transformation have been proposed (reviewed by Specht & Bartlett [68]). Some recent hypotheses are based on the discovery that homologues of floral homeotic genes are present in gymnosperms. In angiosperms, the ABC model postulates that the different expression patterns of class A, B and C floral homeotic genes give rise to the different floral organs (sepals, petals, stamens and carpels). In *A. thaliana*, class A genes are expressed in whorls 1 (sepals) and 2 (petals); class B genes are expressed in whorls 2 (petals) and 3 (stamens); and class C genes are expressed in whorls 3 (stamens) and 4 (carpel). In gymnosperms, which lack the whorled floral structure found in angiosperms, classes B and C are expressed in reproductive structures. Although class C genes are expressed in both male and female reproductive organs, class B genes are exclusively expressed in the male structures [69]. According to the out of male (or out of female) hypothesis [70], changes in the spatial expression pattern of class B genes in male (or female) cones could have given rise to the hermaphroditic precursors of flowers.

Floral homeotic genes are central to the specification of flower organ identities and were probably a major driver of flower evolution: the 'sliding boundary' [71] and the 'fading borders' [72] models propose that changes in the spatial expression domains of homeotic genes result in gradual transitions in floral morphology. Different floral organs could also potentially arise through changes in the protein interactions of floral homeotic genes or in the promoters of their target genes [73]. Another factor that has promoted flower evolution was the multiple evolution of a floral axis of asymmetry (zygomorphy) in different plant lineages. Interestingly, a mechanism involving the TCP transcription factor CYCLOIDEA was independently recruited to establish bilateral symmetry in several eudicot families [74].

(e) *Evolution of transcription factors and regulatory pathways*

The sequencing of different plant genomes over the last 10 years has opened the door for powerful comparative genomic analyses [75,76]. In particular, the genome of the moss *P. patens*, a basal land plant, and of different chlorophyte algae have greatly increased the evolutionary window open for comparative studies. One of the important outcomes was the discovery that many genes that regulate development are highly conserved in land plants. Nearly all the 50–60 transcription factor gene families found in angiosperms are present in basal land plants (mosses), but only 15–30 of these are present in chlorophyte algae [77,78]. This indicates that there was a large increase in the number of transcription factor families during the first stages of streptophyte evolution (before mosses evolved), but that a core set of transcription factors is highly conserved in land plants. Nevertheless, the average size of each transcription factor family is substantially smaller in mosses (less than 10 genes per transcription factor family) than in angiosperms (20–25 genes per family) [78]. This suggests that there was an expansion and diversification of the different transcription factor families on land, a process probably directly related to the elaboration of the land plant body. Interestingly, despite having a smaller set of transcription factors, mosses appear to have more elaborated two-component signalling systems (involving histidine kinases and response regulators) than angiosperms [76]. The increase in the complexity of plant transcription factor families is reminiscent of the evolution of transcription factors in metazoans: a wide range of transcription factor families and classes are present in demosponges (the most basal metazoan group), but not in choanoflagellates (unicellular organisms that are the sister group to metazoans) [79,80]. The ancestors of bilaterians and cnidarians later underwent an expansion and diversification of transcription factor families, which correlates with an increase in morphological and cell type complexity [79,80].

A major factor driving transcription factor evolution in plants is the frequent occurrence of gene duplications, particularly whole-genome duplications through auto-polyploidy and allopolyploidy events. Many land plant species are polyploid [81], and possibly almost all angiosperm species (including *A. thaliana*) are paleo-polyploids, i.e. diploids with polyploid ancestors. The detection of collinearity between triplicate regions in rosid and asterid species suggests that there was a hexaploidy event in the common ancestor of the main eudicot lineages, around 150 Ma [82]. Additional duplications later occurred independently in several lineages, including two duplications in Brassicales ancestors of *A. thaliana* [83]. It is estimated that the three whole-genome duplications are directly responsible for the generation of 60 per cent of the *Arabidopsis* genes during the last 150 Myr [83,84]. Regulatory genes (including genes involved in transcription and signal transduction) are preferentially retained after large-scale duplication events than after small-scale duplications, probably because of dosage effects and the importance of maintaining a correct stoichiometric balance in protein complexes [84,85]. The three whole-genome duplication events are calculated to be responsible for 90 per cent of the *Arabidopsis* transcription factors created over the last 150 Myr [84]. Interestingly, many whole-genome duplications occurred independently in several plant groups during the Cretaceous–Tertiary boundary 65 Ma, a period of mass extinctions followed by extensive radiations [86]. This suggests that whole-genome duplications may confer a competitive advantage under changing environments and enhance the diversification potential of a lineage [87]. Gene duplication can fuel evolution because a duplicate copy is free to evolve a novel function (neofunctionalization) without compromising the function of the original gene. However, most retained duplicates probably undergo a sub-functionalization process instead, in which complementary loss-of-function mutations occur such that both genes are required to produce the full complement of functions of the single ancestral gene [88].

Mechanisms of gene regulation, by RNA silencing, are partially conserved in land plants. The microRNA (miRNA) machinery appears to have evolved independently in animals and in plants [89]. In plants, miRNAs have been identified in the unicellular chlorophyte *Chlamydomonas*, but these are not homologous to any land plant miRNA [90,91]. Dozens of miRNA families have been identified in land plants [89]. At least 16 of these miRNA families were present in the common ancestor of mosses and vascular plants and are highly conserved in other land plants [92]. However, the majority of miRNAs are lineage-specific and non-conserved [93]. In contrast to miRNAs, short-interfering RNAs (siRNAs) are present in most eukaryotic genomes that have been sequenced to date, implying that they would have been present in the genome of the common ancestor of all plants; accordingly, they have been identified in chlorophytes, mosses and angiosperms [91,94]. The plant-specific class of *trans*-acting siRNA (ta-siRNA) is present in mosses [95] and, less clearly, in *Chlamydomonas* [91].

The major components of auxin signalling in land plants (AUX-IAA, ARFs and TIR1-AFBs) are not present in chlorophyte algae [96,97], suggesting that they evolved in the streptophyte lineage. Nevertheless, some proteins required for auxin synthesis and metabolism are encoded in the genomes of chlorophyte algae [98]. The auxin-signalling response is functional in mosses [99,100] and auxin polar transport has been found to occur in different moss structures [101–104]. However, *Physcomitrella* PIN proteins are functionally related to the PIN5-type proteins that regulate subcellular homeostasis of auxin, and not to the PIN1-type proteins that are responsible for auxin efflux from cell to cell in angiosperms [105]. Furthermore, the expression of *SHI* genes, which regulate auxin biosynthesis, coincides with the sites of auxin response [106]. This suggests that local auxin biosynthesis plays a key role in auxin peak formation in mosses and that PIN-mediated polar transport of auxin only became a key signalling mechanism during the evolution of vascular plants. The amount of active free auxin in plant tissues is determined by a homeostatic mechanism that modulates its synthesis, destruction and conjugation (the formation of inactive auxin–amino acid or auxin–peptide conjugates). One conjugation mechanism involves an adenylation of auxin prior to conjugation. This adenylation reaction is catalysed by GH3 proteins in both *A. thaliana* and *P. patens* [107,108]. The conserved function of GH3 proteins in land plants suggests that the mechanism is ancient and may have been present in early land plants.

All genetic components required for cytokinin signalling are also present in mosses but not in chlorophytes [109]. Nevertheless, there was an expansion of most of the gene families encoding proteins involved in cytokinin signalling in vascular plants [109]. There are candidate gibberellin (GA) biosynthetic genes and GA–DELLA signalling components [110–113] in the *P. patens* genome, but there is no functional evidence for the existence of a GA-dependent GID1–DELLA signalling. This mechanism appears to have evolved in vascular plants [111,113], while the characteristic DELLA–GA-mediated growth restraint probably evolved among monilophytes and seed plants after the divergence of lycophytes [113]. This suggests that the GA-signalling pathways evolved gradually in land plants. An abscisic acid (ABA) signalling response is present in mosses [114,115] and, accordingly, the genome of *P. patens* encodes homologues of receptors and transcription factors involved in ABA signalling [76]. Strigolactones, which regulate shoot branching and biotic interactions in angiosperms, are synthesized and control gametophytic development in mosses [116]. The analysis of the *P. patens* genome suggests that signalling through jasmonic acid, ethylene or brassinosteroids evolved after the divergence of mosses from other land plants [76]. Nevertheless, we should be aware that most of the evidence used to infer the evolutionary origin of signalling pathways is based on the genomic identification of homologues of known biosynthetic enzymes, receptors or signal transducers; it is possible that independent plant lineages have evolved slightly different signalling pathways, and it will take more than comparative genomics to identify these mechanisms.

3. GENETIC 'BRICOLAGE'

One of the major breakthroughs achieved by evolutionary developmental biology over the last two decades was the discovery that the genetic tool kits of distantly related animals are remarkably similar. Form evolves largely by altering the expression of functionally conserved proteins, usually through mutations in the *cis*-regulatory regions of regulatory genes and their targets [117–120], although there are a few examples where changes in the primary structure of a protein have resulted in changes in function [121,122]. Recent functional and genomic studies in non-angiosperm model systems are demonstrating that the same principle holds true in plants. Many angiosperm transcription factors and regulatory genes have homologues in early diverging land plants [77,78,123], and large families such as the homeobox and basic-helix-loop-helix proteins underwent large diversifications before the separation of the major land plant lineages [47,124]. This indicates that despite the dynamic character of plant genomes that undergo frequent duplications and gene losses, ancient lineages of regulatory genes were preserved throughout land plant evolution.

Since regulatory genes are often more ancient than the morphological structures that they control (e.g. floral homeotic gene families are older than angiosperm flowers), it is likely that regulatory genes were recruited from pre-existing functions to control the development of novel structures. Interestingly, structures independently controlled by homologous regulatory genes often have similar functions. For example, the KNOX–ARP mechanism controls megaphyll development in angiosperms and microphyll development in lycophytes, despite the independent origins of megaphyll and microphylls in vascular plants [59]; a possible explanation for this convergence is that leaf evolution was constrained by an ancestral mechanism that controlled branching. This would mean that the recruitment of regulatory genes to new functions could be constrained by the ancestral function of those genes. Likewise, the formation of boundary domains that delimit the leaflets of compound leaves is promoted by *NAM/CUC3* genes in several eudicots, despite the multiple origins of compound leaves [125], and *RSL* genes control the differentiation of root hairs in angiosperms and rhizoid filaments in mosses [45]. Similar examples were found in metazoans, where the development of a variety of non-homologous animal appendages is controlled by Distal-less/Dlx homeoproteins [126], the development of the different types of eyes is controlled by an array of homologous transcription factors (reviewed by Vopalensky & Kozmik [127]), and the development of different types of hearts is governed by a set of homologous transcription factors in different animals (reviewed by Olson [128]). This dissociation between homology at the genetic level and analogy at the morphological level has been termed deep homology [119,129]. The concept reflects the fact that homology depends on the hierarchical level that is being compared: for example, the wings of birds and bats are not homologous as wings, because wings evolved independently in the two lineages, but they are homologous as tetrapod forelimbs. Similarly, two structures may be analogous in a morphological context, but (deeply) homologous at a genetic level [129,130].

The recruitment of ancient regulatory genes and networks to control the development of novel morphological functions highlights the modular nature of evolution at the molecular level. The earliest land plants had in place a tool kit with homologues of most modern angiosperm-regulatory genes [123]. Although gene duplications and protein evolution were also important sources of genetic innovations during land plant evolution, the genetic 'bricolage' that involved both the reuse (co-option) and reassembly of ancient genetic networks during the last 500 Myr was probably a major driver for the large diversification of plant morphologies on land.

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