Tansley review

Several developmental and morphogenetic factors govern the evolution of stomatal patterning in land plants

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Summary

We evaluate stomatal development in terms of its primary morphogenetic factors and place it in a phylogenetic context, including clarification of the contrasting specialist terms that are used by different sets of researchers. The genetic and structural bases for stomatal development are well conserved and increasingly well understood in extant taxa, but many phylogenetically crucial plant lineages are known only from fossils, in which it is problematic to infer development. For example, specialized lateral subsidiary cells that occur adjacent to the guard cells in some taxa can be derived either from the same cell lineage as the guard cells or from an adjacent cell file. A potentially key factor in land-plant evolution is the presence (mesogenous type) or absence (perigenous type) of at least one asymmetric division in the cell lineage leading to the guardmother cell. However, the question whether perigenous or mesogenous development is ancestral in land plants cannot yet be answered definitively based on existing data. Establishment of ‘fossil fingerprints’ as developmental markers is critical for understanding the evolution of stomatal patterning. Long cell–short cell alternation in the developing leaf epidermis indicates that the stomata are derived from an asymmetric mitosis. Other potential developmental markers include nonrandom stomatal orientation and a range of variation in relative sizes of epidermal cells. Records of occasional giant stomata in fossil bennettites could indicate development of a similar type to early-divergent angiosperms.
I. Introduction

Stomatal pores in the aerial epidermis not only possess immense ecological significance but also represent one of the most consistent micromorphological features in the evolutionary history of land plants (Bateman et al., 1998; Raven, 2002; Ligrone et al., 2012a,b; McAdam & Brodribb, 2012). Stomata occur in the majority of land plants, almost exclusively on the sporophyte, although stomata have occasionally been reported on the gametophyte (prothallus) of the extant model fern *Ceratopteris* (Mahabale, 1947; Pant, 1965) and on gametophytes of some Rhynie Chert polysporangiophytes (Edwards et al., 1998; Taylor et al., 2009). Almost invariably among post-Devonian embryophytes, each individual stoma consists of a symmetric pair of guard cells delimiting a central pore (Sack, 1987; Ziegler, 1987; Edwards et al., 1998), although rare exceptions include the moss *Funaria*. By contrast, the stomatal complex – which by definition encompasses the guard cells plus neighbouring epidermal cells – can differ considerably among taxa. The plethora of complex and often partially overlapping terms surrounding stomata is summarized in Box 1 (Glossary), which is crucial to the understanding of this paper.

The diverse range of stomatal patterning that occurs in different major groups of land plants, both extant and extinct, is described in Table 1. Contrasting stomatal patterns are genetically determined and also apparently characterize major clades (e.g. Carpenter, 2005), although detailed comparative data are at best undesirably patchy. Thus, the stomatal complex could represent a key character in understanding land-plant evolution. Furthermore, at least some of the genes controlling stomatal development are well conserved across land plants (Peterson et al., 2010). In *Arabidopsis*, stomatal development is regulated by a complex signalling cascade of several genes, especially from two major gene families: the basic-helix-loophelix (bHLH) family and the ERECTA family of leucine-rich repeat receptor-like kinases (LRR-RLKS). Three closely related bHLH transcription factors control successive steps in the developmental series: SPEECHLESS (*SPE*) regulates asymmetric divisions, MUTE specifies guard-mother cell (GMC) identity, and FAMA promotes the ultimate (invariably symmetric) GMC division that forms the paired guard cells (Ohashi-Ito & Bergmann, 2006; MacAlister & Bergmann, 2011). In terms of the phylogenetic relationships of these bHLH genes, existing evidence suggests that FAMA is closest to the ancestral form (MacAlister & Bergmann, 2011; Serna, 2011).

Given the large numbers of extinct lineages, understanding land-plant evolution requires detailed comparative studies of (ideally anatomically preserved) fossil plants. Unfortunately, current resolution of the phylogenetic relationships of the major land-plant groups remains imperfect (cf. Bateman et al., 2006; Doyle, 2006; Hilton & Bateman, 2006; Graham & Iles, 2009). Current understanding of the phylogenetic relationships among these groups is summarized in Fig. 1, contrasting the results obtained from morphological and molecular data. There exists serious conflict between molecular phylogenies, which rely solely on extant taxa, and morphological phylogenies, which include fossils. In particular, there is a stark choice between large numbers of characters (molecular data) and considerably greater taxon sampling (morphology; Bateman et al., 2006). Furthermore, as with many studies of development, there exists an inevitable conflict between static atemporal observations and dynamic temporal observations. Thus, it is notoriously problematic to infer development from either fossil cuticles (e.g. Cleal & Shute, 2012) or mature stomatal patterns observed in extant species (e.g. Payne, 1979). Barbacka & Bóka (2000) presented a rare example of fossil stomatal ontogeny in an extinct Jurassic pteridosperm leaf, *Sagenopteris*, representing an order of gymnosperms (Caytoniales) that is placed as sister to angiosperms in many morphological cladistic analyses (Fig. 1). Their study provided a tantalizing clue that hints at the strong potential for further similar studies, but unfortunately it focused primarily on the final stage of stomatal development – specifically, the symmetric division of the GMC – rather than the crucial earlier patterning of the stomatal complex.

Stomatal development has been studied since the early work of Prantl (1872; for a review of early literature see Pant, 1965), and was extensively inferred in fossil seed plants by the influential Swedish palaeobotanist Rudolf Florin. Florin (1933, 1951) famously identified two contrasting patterns of stomatal complexes in gymnosperms (both fossil and extant), which he termed haplochelic and syndetocheilic. These terms were specifically intended to incorporate developmental information (for definitions see Box 1). Florin inferred developmental pathways in fossils by comparing them with extant taxa in which ontogeny was supposedly well known. However, more recent studies of seed-plant fossils prefer the purely descriptive terms anomocytic and paracytic, in order to (at least notionally) avoid problematic assumptions about development – a rationale that has been explicit in morphological cladistic analyses (e.g. Doyle, 1996). Florin (1931, 1933, 1950) also employed the now standardized mesogenous–perigenous terminology for stomatal development (summarized by Pant, 1979; Payne, 1979; for updated definitions see Box 1 and Fig. 2). Thus, Florin controversially considered haplochelic stomata to be both perigenous and anomocytic, and syndetocheilic stomata to be both mesogenous and paracytic. This hypothetical precise correspondence between static and dynamic terms has been extensively challenged in the neobotanical literature (e.g. Pant, 1965; Payne 1979). Unfortunately, no ‘fossil fingerprints’ of developmental regulation have yet been identified to confirm these inferences of stomatal patterning, in contrast with the more convincing ‘fingerprints’ provided by the swirls of tracheids that denote polar auxin transport in fossil woods (Sanders et al., 2007) and the readily visible proximal–distal microspore polarity transition that originated early in seed-plant evolution (Rudall & Bateman, 2007).

Based on comparison of a wide range of literature from palaeobotany and neobotany to developmental genetics, we reassess stomatal diversity in terms of its primary morphogenetic factors and place it in a phylogenetic context. Our goals are to provide insights regarding the evolution of stomatal patterning, and hence the evolution of land plants in general, and to review data that suggest possible developmental mechanisms and potential ‘fossil
### Box 1 Glossary of stomatal terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Cells</td>
<td></td>
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<tr>
<td>Guard cell</td>
<td>One of a pair of specialized epidermal cells that together delimit the stomatal pore. Guard cells are of necessity equal and opposite each other for the stoma to function physiologically; they are formed by a symmetric division</td>
</tr>
<tr>
<td>Guard-mother cell (GMC)</td>
<td>Final stomatal precursor cell; divides symmetrically to form a pair of guard cells</td>
</tr>
<tr>
<td>Meristemoid</td>
<td>Isolated and densely protoplasmic cell that represents a centre of reactivated embryonic activity for tissue differentiation. A meristemoid is usually – although not invariably – the smaller daughter cell that results from an asymmetric division (Bünning, 1952). This term is contrastingly defined in the literature</td>
</tr>
<tr>
<td>Mesogene cell</td>
<td>Neighbour cell that is derived from a precursor cell in the same cell lineage as the GMC, following an asymmetric division (Fryns-Claessens &amp; Van Cotthem, 1973; Payne, 1979). A mesogene neighbour cell is sometimes termed a stomatal-lineage ground cell (SLGC)</td>
</tr>
<tr>
<td>Neighbour cell</td>
<td>Epidermal cell that is located immediately adjacent to a guard cell</td>
</tr>
<tr>
<td>Pavement cell</td>
<td>Unspecialized mature epidermal cell</td>
</tr>
<tr>
<td>Perigene cell</td>
<td>Neighbour cell that is not derived from the same cell lineage as the guard cells (Fryns-Claessens &amp; Van Cotthem, 1973; Payne, 1979)</td>
</tr>
<tr>
<td>Protodermal cell</td>
<td>Developing (undifferentiated) epidermal cell</td>
</tr>
<tr>
<td>Stoma (plural stomata)</td>
<td>Pair of guard cells plus the central pore that they delimit</td>
</tr>
<tr>
<td>Stomatal complex</td>
<td>Close-packed group of stomata that share a single substomatal chamber (although individual stomata are still spaced at least one cell apart)</td>
</tr>
<tr>
<td>Stomatal-lineage ground cell (SLGC)</td>
<td>Larger daughter cell resulting from an asymmetric division of a stomatal precursor cell (Lau &amp; Bergmann, 2012). So that at least one of the neighbour cells is a mesogene cell</td>
</tr>
</tbody>
</table>

| Terms applied to mature stomata (Florin, 1933, 1951; Payne, 1979; Wilkinson, 1979; Kerp, 1990; Carpenter, 2005) |
|-------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Anisocytic                                       | Surrounded by three subsidiary cells, of which one is smaller than the other two                                                        |
| Anomocytic                                       | Lacking subsidiary cells (sometimes termed acyclic)                                                                                       |
| Haplocheilic ('simple-lipped')                  | Term applied to fossil stomata, indicating that the guard cells are derived from a protodermal cell that functioned directly as a GMC, so that all the neighbour cells are perigenous |
| Paracytic                                         | Possessing one or more pairs of lateral subsidiary cells oriented parallel with the guard cells                                           |
| Stephanocytic (including actinocytic, amphichytic, cyclocytic and tetracytic) | Possessing an encircling ring (rosette) of four or more subsidiary cells; subsidiary cells often weakly differentiated from other epidermal cells (indistinct from anomocytic types), or sometimes radially elongated (actinocytic); subsidiary cells sometimes in two rings (stephanocytic–bicyclic) |
| Syndetocheilic ('complex-lipped')               | Term applied to fossil stomata, indicating that the guard cells are derived from a protodermal cell that divided asymmetrically (at least once) to form a GMC and a SLGC, so that at least one of the neighbour cells is a mesogene cell |

| Terms used for stomatal development (Pant & Mehra, 1964a; Pant, 1965; Fryns-Claessens & Van Cotthem, 1973; Payne, 1979) |
|------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Mesogenous (Fig. 2d)                            | Refers to a stomatal complex in which all the neighbouring cells are formed from the same meristemoid as the GMC, following one or more asymmetric divisions |
| Mesoperigenous (Figs 2a[d–f],b,c,3c,d)           | Refers to a stomatal complex with both mesogenous and perigenous neighbouring cells                                                      |
| Perigenous (Figs 2a[b,c],3a,b)                  | Refers to a stomatal complex in which the GMC and all the neighbouring cells are formed from different protodermal cells; sometimes asymmetric divisions occur in neighbouring cells, but not in the cell that gives rise to the GMC |

| Leaf growth, epidermal pre-patterning and cell divisions in stomatal lineage |
|------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Amplifying division                           | Asymmetric division of a meristemoid that creates two daughter cells: an SLGC and a meristemoid or GMC (Lau & Bergmann, 2012)     |
| Asymmetric division                           | Highly polarized mitosis. Intrinsic asymmetric divisions result in daughter cells of different sizes and fates, by unequal segregation of determinants such as spindle orientation and the nuclear cytoskeleton. By contrast, extrinsic asymmetric divisions result in daughter cells that are initially equal but have different fates as a result of differential intercellular signalling, usually because they adopt relative locations that are proximal and distal to the source of the signal, for example in a linear cell file (Abrasch & Bergmann, 2009). Intrinsic and extrinsic asymmetric divisions rely on different relative influences of cell lineages and intercellular signalling, for which there exists a fine balance. We focus primarily on intrinsic asymmetric divisions, because they are observable during studies of development and have been used to characterize contrasting developmental types |
| Diffuse growth                                | Leaf development in which the tissues expand with diffuse meristematic activity, resulting in groups of stomata at different stages of development (Payne, 1979) |
| Lateral division                              | Division of neighbour cell, either symmetric or asymmetric                                                                             |
| Linear pre-patterning                         | Protodermal cell arrangement in linear files, resulting from development where each cell division is parallel with the previous division |
| Rectate growth                                | Leaf development proceeding from an intercalary or marginal meristem, so that all cells at a given distance from the meristem have reached similar stages of development (Payne, 1979) |
| Squared pre-patterning                        | Protodermal cells in groups of four, roughly arranged in a square or rectangle, resulting from development where each cell divides symmetrically across its narrowest width, usually perpendicular to the previous division (Rudall & Knowles, 2013) |
| Spacing division                              | Asymmetric division of an SLGC that creates another meristemoid                                                                            |
fingerprints’ of developmental regulation of stomata. We outline some fundamental principles of stomatal patterning, such as one-cell spacing (Section II) and paired guard cells (Section III), including exceptional cases that suggest possible underlying constraints on patterning. We discuss the influence of leaf development on stomatal patterning and orientation (Section V), and assess the phylogenetic distributions of lateral subsidiary cells (Section VI) and the amplifying divisions that occur in the well-characterized developmental pathway of Arabidopsis (Section VII). An important issue is to evaluate the potentially key roles of highly polarized (asymmetric) mitoses (Section IV), and discuss whether they are ancestral or derived in land-plant evolution, a debate that is intrinsic both to Florin’s (1933) widely applied terminology for fossil seed plants and also to understanding the evolution of developmental-genetic pathways. From the zygote to the embryo and beyond, asymmetric mitoses are significant in the generation of cellular diversity and patterning in plants (Bünnig, 1952). For example, asymmetric mitoses that occur during microspore development are important for differentiation of the generative cell that ultimately produces the paired sperm nuclei (Twell et al., 1998; Rudall & Bateman, 2007). Indeed, there are some close similarities between microspore development and stomatal development, in which the GMC can be closely analogized with the generative cell and the guard cells with the sperm.

Another fundamental aspect of this review is to clarify the contrasting specialist terms that are used by different sets of researchers, including neobotanists, palaeobotanists and developmental geneticists. We update and clarify the complex terminology surrounding both fossil and extant stomata, which we believe employs some circular logic in attempting to combine descriptive and developmental observations. At least some of the existing terminology begins to unravel under close scrutiny, including the supposedly crucial distinction between perigenous and mesogenous (or mesoperigenous) development. For example, Johnson & Riding (1981) noted that the stomatal complex in the conifer Pinus (Fig. 4f) cannot legitimately be termed perigenous (or haplochei-lic), as suggested by Florin (1931, 1933), because the GMC and one of its polar subsidiary cells are derived from the same cell, following an asymmetric division. Payne (1979) even argued that totally perigenous stomata do not exist, at least in flowering plants, because a GMC is almost always derived from an asymmetric division, and therefore one of the neighbouring cells is by definition its sister cell. Yet, recent observations on early-divergent extant angiosperms (Rudall & Knowles, 2013) have shown that stomatal development can occur without asymmetric divisions, at least in some taxa, and development is apparently exclusively perigenous in water-lilies.

II. One-cell spacing

Spacing represents a fundamental principle of stomatal patterning on leaves. The guard-cell pairs of most land plants (Figs 4–7) are typically separated from each other by at least one pavement epidermal cell, thereby following the one-cell spacing rule sensu Sachs (1991). Even the stomata of the Silurian and Lower Devonian rhyzophytes, which had bifurcating leafless axes, were spaced apart from each other on the plant surface (Edwards et al., 1998). One-cell spacing occurs even in taxa where stomata are clustered closely together in ‘islands’ that share a single underlying stomatal chamber, such as Begonia (Tang et al., 2002) and Cinnamomum (Zhao et al., 2006). It is likely that one-cell spacing results partly from inhibition effects caused by cell signalling during development (e.g. Korn, 1993) and partly from controlled asymmetric cell divisions that orient sister stomatal complexes away from each other within a single cell lineage (Sachs, 1994; Hara et al., 2007; Serna, 2009). An early debate focused on the relative influences of cell lineages and intercellular signalling during development, a fine balance that could shift between different plant groups (Sachs, 1994). Croxdale’s (2000) ‘unified theory’ of stomatal patterning suggested that epidermal cell fate is determined by the appropriate intercellular signalling occurring at a particular point in the cell-division cycle, thus combining two major factors: cell lineages and cell interactions.

The reasons for the almost universal adherence to one-cell spacing remain obscure; the most likely rationale is the polarized divisions that ensure that a densely protoplasmic meristemoid lies adjacent to its larger sister cell (Section IV). Exceptions to the one-cell spacing rule can occur in the stomata of some early-divergent land-plant lineages such as hornworts (Ziegler, 1987) and the frequently contiguous stomata in adjacent cell files on leaves of Selaginella (Pant & Mehra, 1964c). Another exception is the aggregated stomata commonly associated with nectaries or hydathodes (Fahn, 1979; Pillitteri et al., 2008). Similar aggregations occur in some well-characterized genetic mutations, such as the too many mouths (tmn) mutant of Arabidopsis (Yang & Sack, 1995; Nadeau & Sack, 2003). The TMM gene, a member of the LRR-RLP family, is present as a single copy in poplar, grass and moss genomes, and could have a well-conserved function to regulate stomatal spacing (Peterson et al., 2010). Another rare mutant in which adjacent stomata are formed in the same axial cell file is ecriferum-g in barley (Hordeum vulgare) (Zeiger & Stebbins, 1972). Thus, the few exceptions to the one-cell spacing rule are phylogenetically diverse and apparently possess few morphogenetic similarities (other than division asymmetry) that could indicate an underlying developmental constraint.

III. Paired guard cells

The stoma sensu stricto almost invariably consists of a symmetric pair of guard cells delimiting a central pore, the most notable exception being the single guard cell that encircles the pore in the moss Funaria (Sack & Paolillo, 1985). In Arabidopsis, two transcription factors FAMA and FOUR LIPS (FLP) regulate the final symmetric division of the GMC to form a pair of guard cells: their loss-of-function mutations result in reiterative symmetric divisions of the GMC (Ohashi-Ito & Bergmann, 2006; Pillitteri et al., 2007).

A possible major exception to the paired guard-cell arrangement occurs in the air pores that characterize the gametophytes of some thallose liverworts; these air pores resemble stomata in location and function (in allowing gas diffusion: Raven, 2002), but have a contrasting cellular arrangement, the pore being demarcated by...
Table 1 Summary of stomatal types in major groups of land plants. Wholly extinct groups (bracketed in Fig. 1) are asterisked. Earlier literature on stomatal ontogeny was reviewed by Pant (1965). This table is intended to be read in conjunction with Figs 3–7.

<table>
<thead>
<tr>
<th>Group</th>
<th>Stomatal Type</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liverworts</td>
<td>Stomata absent, although gametophyte air pores sometimes homologized with stomata</td>
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<tr>
<td>Hornworts</td>
<td>Stomata anomocytic; development probably perigenous but requires review</td>
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<td></td>
<td>Stomata occur in the sporophytes of some extant hornworts (e.g. <em>Anthoceros</em> and <em>Phaeaceros</em>); they lack subsidiary cells, so are anomocytic and sometimes contiguous (Bower, 1935; Pant, 1965; Ziegler, 1987). Development is reportedly perigenous (Bower, 1935; Pant, 1965). Hornwort stomata do not open and close (Lucas &amp; Renzaglia, 2002)</td>
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</tr>
<tr>
<td>Mosses</td>
<td>Stomata anomocytic; development probably perigenous but requires review</td>
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<td></td>
<td>Stomata occur on the sporophyte of some mosses, typically on the lower part of the capsule. Duckett et al. (2009) questioned the homology of the pseudostomata of <em>Sphagnum</em> (see also Haig, 2013). Moss stomata lack subsidiary cells, so are anomocytic (Paton, 1957; Ziegler, 1987). In <em>Funaria</em>, the guard-mother cell (GMC) fails to divide, so only a single guard cell is produced (Sack &amp; Paolillo, 1985). Moss stomata are widely described as perigenous (e.g. Pant, 1965; Väätäinen &amp; Bergmann, 2012), but this interpretation was challenged by Payne (1979) who considered them to be mesoperigenous, citing unpublished data on <em>Lycopodium</em> and <em>Selaginella</em></td>
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<tr>
<td>Rhiophytes*</td>
<td>Stomata anomocytic; development unknown</td>
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<tr>
<td></td>
<td>Stomata of extinct early land plants from the Silurian and Lower Devonian Rhynie Chert (including rhiophytes) are invariably anomocytic (Ziegler, 1987; Edwards et al., 1998)</td>
<td></td>
</tr>
<tr>
<td>Lycophytes (lycopsids plus zosterophylls*)</td>
<td>Stomata anomocytic; development probably perigenous but requires review</td>
<td></td>
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<tr>
<td></td>
<td>Lycopsid stomata are anomocytic and axially oriented, developing basipetally on the leaf (Pant &amp; Mehra, 1964c; Ziegler, 1987). Development is perigenous in the lycopsids <em>Lycopodium</em>, <em>Selaginella</em> and <em>Isoetes</em> (Pant &amp; Mehra, 1964c; see also Brown &amp; Lemmon, 1985), although this interpretation was challenged by Payne (1979), because the divisions that give rise to the GMC sometimes appear slightly asymmetric (e.g. <em>Selaginella</em>; Rieber, 1925).</td>
<td></td>
</tr>
<tr>
<td>Trimerophytes* (paraphyletic; e.g. <em>Psilophyton</em>)</td>
<td>Stomata anomocytic; development unknown</td>
<td></td>
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<tr>
<td></td>
<td>Plants lacked leaves, and had anomocytic stomata with three to five neighbour cells arranged longitudinally along the axis (Zdeb'ska, 1972)</td>
<td></td>
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<tr>
<td>Psilotaceae (<em>Psilotum</em>, Fig. 4a; <em>Tmesipteris</em>)</td>
<td>Mature stomata are anomocytic (Fig. 4a); Bower (1935) noted that they resemble stomata of <em>Rhynia</em>. Development in <em>Tmesipteris</em> was described and illustrated as perigenous by Marót (1966) and Pant &amp; Khare (1971), and asymmetric divisions were not recorded in <em>Psilotum</em> by Pant &amp; Mehra (1963) or Mickle et al. (2012). Payne (1979) challenged this interpretation (see text)</td>
<td></td>
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<tr>
<td>Ophioglossaceae (Ophioglossum)</td>
<td>Mature stomata are anomocytic; development could be either mesoperigenous or perigenous; requires review</td>
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<td></td>
<td>Mature stomata are anomocytic and contrastingly reported as perigenous (Pant &amp; Khare, 1969; Thurston, 1969; Van Cotthem, 1970; Parrot &amp; Got, 1985; Ziegler, 1987). <em>Marattia</em> (1965; Pant &amp; Khare, 1969; Marót, 1966) described the division of the stomatal precursor cell as asymmetric (or occasionally) symmetric; the GMC is usually the smaller and more distal daughter cell</td>
<td></td>
</tr>
<tr>
<td>Equisetales (Equisetum)</td>
<td>Stomata paracytic, with two mesogene subsidiary cells overlying the guard cells; development mesogenous, but of an unusual type</td>
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<tr>
<td></td>
<td>The overlying subsidiary cells possess radiating ridges on the wall adjacent to the guard cell (e.g. Hauke, 1957). <em>Equisetum</em> is unusual in that, within axial cell files, each precursor cell divides once asymmetrically, then the larger daughter cell divides again, resulting in a trio of cells: an inner GMC and a lateral pair of mesogene subsidiary cells (Chatterjee, 1964; Pant &amp; Mehra, 1964c; Pant &amp; Kidwai, 1968; Dayanandan &amp; Kauffman, 1973)</td>
<td></td>
</tr>
<tr>
<td>Marattiales</td>
<td>Stomata stephanocytic (cyclocytic) or anomocytic; development mesoperigenous</td>
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<td></td>
<td>Stomata of the extant genus <em>Angiopteris</em> are oriented regularly on pinnae, parallel with veins; they possess one or two encircling rings of subsidiary cells (e.g. Pant &amp; Khare, 1969), and are sometimes described as a mixture of anisocytic, cyclocytic, tetracytic and rarely anomocytic (Rolleri et al., 1991). Fossil Marattiales (e.g. <em>Pecopteris</em> and <em>Seitenberga</em>) are stephanocytic (cyclocytic) or anomocytic (Pieniščka &amp; Bek, 2003; Pieniščka et al., 2005)</td>
<td></td>
</tr>
<tr>
<td>Polypodieae (‘fem’ sensu stricto, Fig. 4b)</td>
<td>Stomata probably ancestrally anomocytic, but many other types present; development probably ancestrally mesoperigenous but requires further review</td>
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</tr>
<tr>
<td></td>
<td>Mature stomata are either anomocytic (e.g. in the earliest-diverging lineage, Osmundaceae) or a range of different types (Van Cotthem, 1970; Ziegler, 1987). Development is mesoperigenous in <em>Osmunda</em> and some other ferns (Marót, 1966; Pant &amp; Khare, 1969), although there are also reports of perigenous stomata in ferns (Pant, 1965; Thurston, 1969; Van Cotthem, 1970; Ziegler, 1987). In <em>Anemia</em> and <em>Pyrrhospia</em>, the stomata appear entirely surrounded by a single subsidiary cell in paradermal and surface view; however, this apparent anomaly can be explained in transverse section as asymmetric division of the stomatal precursor cell at a right-angle plane, so that the (smaller) outer cell is the GMC, which subsequently divides normally into two guard cells (Pant, 1965; Marót, 1966). In many ferns, the SGCL further divides asymmetrically to form two or more U-shaped subsidiary cells at one stomatal pole (Sen &amp; De, 1992)</td>
<td></td>
</tr>
<tr>
<td>Progymnosperms* (paraphyletic)</td>
<td>Stomata anomocytic; development unknown</td>
<td></td>
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<td></td>
<td>Stomata of <em>Archaeopteris</em> have been described as ‘simple’ (anomocytic; Carluccio et al., 1966); stomata of <em>Cecropis</em> as anomocytic (Stubbfield &amp; Rothwell, 1989); stomata of <em>Tanaitis</em> as ‘longitudinal and elliptical, surrounded by 5–6 fusiform cells’ (Krasiliov et al., 1987). Stomatal features in Aneurophylales are unknown</td>
<td></td>
</tr>
<tr>
<td>Palaeozoic pteridosperms* (paraphyletic; <em>Cyclopteris</em>, Fig. 5–a–c)</td>
<td>Mature stomata were interpreted as haplocheilic (anomocytic) by Florin (1933), which was later generalized as anomocytic. Occasional reports of paracytic stomata in Palaeozoic seed-ferns (e.g. <em>Alethopteris</em>: Stidd, 1988; <em>Neuralithopteris</em>: Cleal &amp; Shute, 1992) were re-interpreted as actinocytic (Reihmann &amp; Schabillon, 1985) or anomocytic (e.g. Doyle &amp; Donoghue, 1986; Doyle, 2006); in fact, both conditions exist within these medullosans (Cleal &amp; Shute, 2012). <em>Medullosa</em> was described as actinocytic (stephanocytic) and haplocheilic by Hamer &amp; Rothwell (1988). <em>Elkinsia</em> was scored as stephanochalic by Carpenter (2005) and as unknown by Doyle (2006). <em>Lyginopteris</em> was scored as anomocytic (haplocheilic) by Doyle (2006). <em>Callistophyton</em> was scored as unknown by Crane (1985) and Doyle (2006)</td>
<td></td>
</tr>
</tbody>
</table>
Table 1 (Continued)

| Taxonomic group | Stomatal development
|-----------------|-----------------------|
| *Corystospermales* (DICRIDIUM, Fig. 5h,i) | Stomata paracytic; development unknown
| *Peltaspermales* (CALLIPTERIS; GLENOPTERIS, Fig. 7) | Stomata anomocytic; development unknown
| *Cycadales* (extant, Fig. 4c; fossil, Fig. 5e,f) | Stomata anomocytic; development probably perigenous but requires review
| *Ginkgoales* (Fig. 4e,f; fossil, Fig. 6h) | Stomata anomocytic/stephanocytic; development incompletely known
| *Ginkgoales* (GINKGO, Fig. 4d) | Stomata anomocytic/stephanocytic; development both perigenous and mesoperigenous
| *Gnetales* | Stomata anomocytic or paracytic; development unknown
| *Pentoxylales* (Fig. 5e) | Stomata anomocytic; development unknown
| *Gnetales* (sister to the remaining extant conifers) | Stomata anomocytic/stephanocytic; development probably perigenous but requires review
| *Pentoxylales* (Fig. 5d) | Stomata anomocytic; development unknown
| *Coniferales* (extant, Fig. 4e,f; fossil, Fig. 6h) | Stomata paracytic; development probably perigenous but requires review
| *Corystospermales* (DICRIDIUM, Fig. 5h,i) | Stomata paracytic; development unknown
| *Peltaspermales* (CALLIPTERIS; GLENOPTERIS, Fig. 7) | Stomata anomocytic; development unknown
| *Cycadales* (extant, Fig. 4c; fossil, Fig. 5e,f) | Stomata were reported as haplocheilic by Florin (1933) and Crane (1985); scored as anomocytic (haplocheilic) by Doyle (2006), and stephanocytic by Carpenter (2005). Perigenous development was reported for Cycas by Pant & Mehra (1964a), but more developmental studies are needed
| *Ginkgoales* (GINKGO, Fig. 4d) | Stomata were scored as anomocytic (haplocheilic) by Doyle (2006), and stephanocytic by Carpenter (2005). Asymmetric divisions were not recorded by Pant & Mehra (1964a); however, Kausik (1974b) reported mesoperigenous stomata, and Rudall et al. (2012) found both perigenous and mesoperigenous stomata in the same leaf (with asymmetric divisions; degree of asymmetry variable); also asymmetric lateral divisions and amplifying divisions
| *Corystospermales* (Fig. 5d) | Stomata anomocytic; development unknown
| *Pentoxylales* (Fig. 5e) | Mature stomata were interpreted as haplocheilic, anomocytic by Florin (1933), and scored as anomocytic (haplocheilic) by Doyle (2006). Stomata in Cordaixylon were described as having two lateral subsidiary cells (Crane, 1985)
| *Coniferales* (extant, Fig. 4e,f; fossil, Fig. 6h) | Stomata were scored as anomalocytic (haplocheilic) by Doyle (2006), and stephanocytic by Carpenter (2005). Stomata were scored as anomocytic (haplocheilic) by Doyle (2006), and stephanocytic by Carpenter (2005). Stomata were scored as anomocytic (haplocheilic) by Doyle (2006). However, a perigenous interpretation was challenged by Payne (1979). Stomatal development in Pinus (sister to the remaining extant conifers) is mesoperigenous (Johnson & Riding, 1981)
| *Corystospermales* (Fig. 5d) | Stomata were scored as anomalocytic (haplocheilic) by Doyle (2006), and stephanocytic by Carpenter (2005). Asymmetric divisions were not recorded by Pant & Mehra (1964a); however, Kausik (1974b) reported mesoperigenous stomata, and Rudall et al. (2012) found both perigenous and mesoperigenous stomata in the same leaf (with asymmetric divisions; degree of asymmetry variable); also asymmetric lateral divisions and amplifying divisions
| *Coniferales* (extant, Fig. 4e,f; fossil, Fig. 6h) | Stomata were scored as anomalocytic (haplocheilic) by Doyle (2006), and stephanocytic by Carpenter (2005). Stomata were scored as anomocytic (haplocheilic) by Doyle (2006), and stephanocytic by Carpenter (2005). Asymmetric divisions were not recorded by Pant & Mehra (1964a); however, Kausik (1974b) reported mesoperigenous stomata, and Rudall et al. (2012) found both perigenous and mesoperigenous stomata in the same leaf (with asymmetric divisions; degree of asymmetry variable); also asymmetric lateral divisions and amplifying divisions

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groups of cells that are often tiered. Some researchers (e.g. Pant, 1965) have regarded the meristemoid that forms the liverwort air pore as homologous to the GMC of stomata, but this hypothesis still requires explicit testing. A stomatal GMC typically divides only once, whereas in liverworts the meristemoid undergoes a series of mitoses. Pant (1965) analogized the liverwort air pore with abnormal stomata that have frequently been recorded in many different taxa, including ferns, cycads and angiosperms, in which one or both guard cells divide further to form a ring of guard cells. He also noted a similarity between liverwort air pores and the distinctive 'bistratose' stomatal pore of *Equisetum* (Table 1), in which a pair of mesogene lateral subsidiary cells overlies a pair of guard cells, together forming a complex pore. These analogies do not imply homology, but are useful indicators that similar anomalies can occur during evolution in contrasting lineages.

**IV. Asymmetric cell divisions**

In terms of stomatal development, a critical factor in determining whether development is mesogenous or perigenous is whether formation of the GMC is preceded by a highly polarized (asymmetric) mitosis. In mesogenous and mesoperigenous
development, an asymmetric division of a stomatal precursor cell produces two daughter cells that differ in size, shape and fate (Fig. 2c). These daughter cells are a (typically smaller) specialized precursor cell (a meristemoid or a GMC) and a (typically larger) stomatal-lineage ground cell (SLGC). Thus, an apparently fundamental question in understanding the evolution of stomatal patterning is whether perigenous or mesoperigenous development is ancestral. Earlier opinions differ on this topic, partly because of the problems of interpreting development in fossils, but perhaps also because of some variation in this character, either in degree of mitotic asymmetry or in relative numbers of symmetric versus asymmetric mitoses.

Put simply, anomocytic stomata (often equated with haplochelic stomata) were traditionally considered to represent the ancestral type, at least among seed plants (Florin, 1933, 1951). Conversely, paracytic stomata (often equated with syndetocheilic stomata and mesogenous/mesoperigenous development) were considered derived. This evolutionary hypothesis was based partly on the widely held assumption that relatively simple morphologies (such as anomocytic stomata) are primitive, and partly because all Palaeozoic seed-plant lineages are anomocytic (Table 1; Fig. 5a–d). Indeed, the shared presence of paracytic stomata is one of the features that group bennettites with angiosperms in morphological cladistic analyses of seed plants (e.g. Doyle & Donoghue, 1986). However, the lateral subsidiary cells of paracytic stomata can be either perigene, as in grasses (Fig. 2b), or mesogene, as in some other angiosperms such as Magnolia (Fig. 2e) and in the early-divergent pteridophyte Equisetum (Table 1).

The question of whether perigenous or mesoperigenous development is ancestral in land plants cannot be answered definitively based on existing data. Our literature review of stomatal patterning across major groups of land plants reveals sparse and sometimes contrasting records within most major groups, and almost no data on development in extinct fossil groups (Table 1). Our phylogenetic optimization tentatively broadly supports the widespread hypothesis that perigenous stomata, which lack asymmetric divisions in the cell lineage that leads to guard-cell formation, represent the ancestral type, regardless of whether morphological or molecular phylogenies are used. Admittedly, the molecular tree could indicate a reversal to the perigenous condition in Psilotum, depending on the interpretation of Ophioglossum (Fig. 1). Stomatal development is probably perigenous in the extant lycophytes Lycopodium, Selaginella and Isoetes (Pant & Mehra, 1964c). However, the divisions that give rise to the GMC sometimes appear slightly asymmetric in these taxa (Riebner, 1925), and stomatal development was reported as mesoperigenous in Lycopodium and Selaginella by Payne (1979, citing unpublished data). Stomata of Equisetum are unusual; development is clearly mesogenous, but of a divergent type that is difficult to compare with those of other taxa.

The alternative theory – that mesoperigenous development is ancestral – was robustly championed by Payne (1979), who reported asymmetric divisions during stomatal development in all early land-plant lineages (Table 1). For example, Payne (1979) reinterpreted development in the extant psilophyte Tmesipteris (sister to Psilotum) as mesoperigenous rather than perigenous, based partly on Maróti’s (1966) images showing a curved wall in the meristemoid-forming division plane. Maróti noted only weak asymmetry in some meristemoid-forming divisions of another phylogenetically critical taxon, Ophioglossum, which represents a basal lineage of ferns. The presence of both perigenous and mesoperigenous stomata in leaves of other phylogenetically significant taxa such as Ginkgo (Rudall et al., 2012) and Amborella (Rudall & Knowles, 2013) indicates that asymmetric division is not essential for stomatal development. Pant’s (1965) ‘arrested branch hypothesis’ also contended that perigenous...
development is the most reduced – and hence evolutionarily most advanced – type.

The bHLH transcription factor SPCH is well conserved in the moss Physcomitrella and the lycophyte Selaginella, as are its subgroup Ia bHLH homologues MUTE and FAMA (MacAlister et al., 2007; MacAlister & Bergmann, 2011). SPCH, MUTE and FAMA together play an essential role in the production of stomata, and SPCH-like sequences are readily identifiable. However, the precise roles of SPCH orthologues in Physcomitrella and Selaginella remain to be determined, a goal that is important in addressing the questions raised in this section. If their role in these phylogenetically pivotal taxa ultimately proves to be similar to the role demonstrated for SPCH in Arabidopsis, in promoting asymmetric cell divisions (MacAlister & Bergmann, 2011), these data would arguably support the hypothesis that perigenous development (lacking asymmetric divisions) represents a derived condition. More work is needed on stomatal development of early land plants, especially the moss Physcomitrella; their stomata are widely described as perigenous (Table 1), but the slight asymmetry of the stomatal precursor division in Selaginella calls into question whether protoplasmic and mitotic polarity are indeed essential for differentiation (Bünnig, 1952).

Regardless of which type of development is ancestral in land plants, the ancestral conditions in progymnosperms and Palaeozoic pteridosperms remain undetermined. These taxa mostly possess anomocytic stomata (Table 1), which could be either mesoperigenous or perigenous (Table 1). Few data exist for cycads, but those that do suggest perigenous development (Pant & Mehra, 1964a). Rudall & Knowles (2013) observed no asymmetric divisions during stomatal development in the water-lilies that they examined, and also found occasional perigenous stomata in Amborella. Indeed, Florin’s hypothesis that anomocytic stomata are equivalent to perigenous (haplocheilic) stomata is apparently correct for water-lilies and cycads, and hence could also be true for Palaeozoic pteridosperms. However, this much-repeated correlation fails for many other groups possessing anomocytic stomata. For example, many seed plants with linear leaves have at least one asymmetric division in the stomatal cell lineage, as in the conifer Pinus (Johnson & Riding, 1981) and liloid monocots such as Lilium (Tomlinson, 1974). In these species, stomata are mesoperigenous; the GMC is formed distal to the SLGC. Thus, it is highly risky to infer stomatal development from fossil cuticles unless clear developmental markers can be observed, such as nonrandom stomatal orientation.

Amborella and water-lilies represent the successive sister taxa to all other flowering plants in most recent molecular phylogenetic analyses (e.g. Graham & Iles, 2009). Rudall & Knowles (2013) hypothesized an evolutionary reversal to perigenous stomata in water-lilies, involving a loss of asymmetric divisions in the stomatal cell lineage. This transition could reflect a loss of SPCH expression at a critical developmental phase, perhaps related to neotenic development of water plants. However, MUTE could also play a significant role; over-expression of MUTE results in pavement cells assuming GMC identity without asymmetric division (Pillitteri et al., 2007). Evolutionary loss of asymmetric divisions has been reported in nonstomatal cell lineages in other angiosperms; for example, a loss of long–short cell alternation probably occurred in both the leaf epidermal silica cells and rhizodermis of rice (Oryza sativa) and its close allies (Kim & Dolan, 2011; Rudall et al., 2013).

V. Leaf development and stomatal orientation

The type of leaf development influences stomatal structure and patterning. Payne (1979) coined the term ‘rectate’ growth for cases where leaf and epidermal development proceeds sequentially from an intercalary or marginal meristem, so that all stomata located at a given distance from the meristem are of similar age and development (e.g. in many monocots, conifers and lycophytes). In these plants, the asymmetric division occurs perpendicular to the axis, so that the resulting GMC is square rather than angular, and is always the most distal of the two daughter cells (i.e. furthest from the meristem). Development is basipetal in lycophytes, monocots and some eudicots, but acropetal in the pteridophyte Ptilium (Pant, 1965). Payne (1979) contrasted rectate growth with diffuse growth, in which the tissues continue to expand with diffuse meristematic activity, resulting in stomata of different ages in the same region. Similarly, Croxdale (2000) contrasted the linear, ‘polarized’ growth of many monocot leaves with the reticulate, ‘patchwork quilt’ pattern of many dicotyledonous angiosperms.

In reticulate-veined angiosperms with ‘squared’ epidermal pre-patterning (Box 1), asymmetric division of a stomatal precursor cell often results in the formation of an angular meristemoid that gives rise to the GMC. In these cases, stomata are oriented randomly. Examples include the archetypal eudicot model organism, Arabidopsis (Zhao & Sack, 1999), together with Amborella (Rudall & Knowles, 2013) – the putative sister to all other extant angiosperms – and many other angiosperms (Table 1; Figs 3, 7). In both Arabidopsis and Amborella, stomatal development is preceded by an asymmetric division, although amplifying divisions are common in Arabidopsis but absent from Amborella. By contrast, in species with epidermal cells arranged in linear files, such as many monocots with linear leaves (Fig. 7a–c) and conifers with needle leaves (Fig. 4e,f), stomata are almost exclusively oriented in the same direction; the GMC division plane (and hence the stomatal pore) is parallel with the leaf axis. All of these taxa are meso(peri)genous; the stomatal precursor cell undergoes asymmetric mitosis, regardless of whether stomatal orientation is random or nonrandom. A significant difference is the type of leaf development and hence the shape of the GMC: angular in Arabidopsis and regular in linear-leaved monocots. Both types occur in Amborella (Fig. 3).

Stomatal orientation is also nonrandom in some ferns and cycads (Fig. 4a–c), water-lilies (Rudall & Knowles, 2013) and many other taxa (Butterfass, 1987). However, in water-lilies, protodermal patterning is squared rather than linear (Box 1); the stomata are initiated almost simultaneously and mature synchronously, rather than sequentially as in monocots (Rudall & Knowles, 2013). The reason that stomatal orientation is so regular in water-lilies is that these taxa apparently lack asymmetric divisions in the stomatal cell lineage, so divisions are mostly aligned with other cells. Thus, nonrandom stomatal orientation has different underlying developmental regulation in different groups.
Another taxonomic group that typically displays characteristic nonrandom stomatal orientation is the gymnosperm order Bennettitales (Table 1; Fig. 6a–f). This diverse group is known only from fossils, and is placed phylogenetically close to the angiosperms in most morphological cladistic analyses (Fig. 1), based partly on the shared presence of paracytic stomata (e.g. Crane, 1985; Doyle & Donoghue, 1986; Doyle, 2006; Hilton & Bateman, 2006). A distinctive feature of the paracytic stomata of many bennettites is their frequently transverse alignment, with their apertures perpendicular to the veins (e.g. Delevoryas & Hope, 1976; Watson & Sincock, 1992). The reason for the unusual transverse orientation of stomata is probably related to the venation patterns. Leaf venation in bennettites is fundamentally linear (Watson & Sincock, 1992), although leaves of some taxa (e.g. Dictyozamites) have reticulate regions. Comparison of the stomata of bennettites with those of some monocots with nonlinear leaves will help to elucidate this issue (e.g. Conover, 1991; P. J. Rudall & E. Chen, unpublished).

VI. Lateral subsidiary cells

Many stomatal complexes include lateral subsidiary cells, organized either as a ring or as a pair on each side of a stoma. Paracytic stomata possess one or more pairs of modified lateral neighbour cells (subsidary cells), and stephanocytic stomata possess a rosette of several distinct subsidiary cells surrounding the guard cells.

Lateral subsidiary cells can be either perigene (resulting from asymmetric mitoses in neighbouring cells) or mesogene (belonging to the same cell lineage as the GMC). Perigene lateral subsidiary cells are common in a phylogenetically broad range of taxa that extends from Ginkgo to grasses. Stomata in Ginkgo are each surrounded by a ring of five to seven narrow lateral cells, at least partly resulting from lateral divisions of neighbour cells (Rudall et al., 2012). Lateral divisions in neighbour cells also occur in the early-divergent angiosperm order Austrobaileyales (Rudall & Knowles, 2013), although they are apparently absent from Amborella and water-lilies.

A single lateral pair of subsidiary cells (i.e. the paracytic condition) represents a characteristic feature of commelinid
monocots such as grasses and *Tradescantia* (Boetsch et al., 1995; Croxdale, 1998). In grasses (e.g. Pickett-Heaps & Northcote, 1966; Gallagher & Smith, 2000) and some grass relatives (e.g. Flagellaria; Sack, 1994), the margins of the lateral subsidiary cells are precisely aligned with the dumbbell-shaped guard cells. Indeed, it has been shown that in grasses lateral subsidiary cells play a physiological role associated with stomatal function (Franks & Farquhar, 2007), and that elevated carbon dioxide affects the patterning of subsidiary cells in *Tradescantia* (Boetsch et al., 1996). The lateral subsidiary cells in the paracytic stomatal complex of grasses and *Tradescantia* are always perigene cells (e.g. Tomlinson, 1974; Croxdale, 1998), even though stomatal development in these taxa is mesoperigenous (because one of the polar contact cells is a mesogene cell or SLGC derived from the same precursor cell as the GMC). Studies of maize (*Zea mays*) (e.g. Facette & Smith, 2012) have shown that the gene *PANGLOSS1* (*PAN1*), a member of the LRR-RLK family, promotes asymmetric division of lateral neigh-
bouring cells to form the characteristic paracytic complex. By contrast, in some other taxa with paracytic stomata, either one or both of the lateral subsidiary cells are mesogene cells (i.e. derived from the same cell as the GMC). This type is common in magnoliids and eudicots. In, for example, in the magnoliid families Annonaceae and Magnoliaceae, stomata are paracytic with two to six lateral subsidiary cells parallel with the guard cells. In at least some species of these families, a rectangular precursor cell divides asymmetrically and the smaller daughter cell (a meristemoid) also divides asymmetrically (Fig. 2e). These divisions result in a triad, of which the central cell is a GMC and the flanking cells are lateral subsidiary cells (Paliwal & Bhandari, 1962; Patel, 1971). The lateral subsidiary cells may then divide further to form more lateral cells. In other taxa, one lateral subsidiary cell is a mesogene cell and the other is a perigene cell (e.g. Payne, 1970; Farooqui, 1982), although it is impossible to distinguish between them in the mature epidermis. The ‘living fossil’ genus *Equisetum*, a member of the non-lycopsid pteridophyte lineage that is paraphyletic in morpho-
logical analyses but monophyletic in molecular analyses (Fig. 1), apparently possesses axial cell files that form meristemoids following asymmetric divisions (Table 1). In *Equisetum*, each meristemoid divides once asymmetrically, then the larger daughter cell divides again, resulting in a triad of cells: an inner GMC and a lateral pair of mesogene subsidiary cells (Pant & Mehra, 1964c; Pant & Kidwai, 1968; Dayanandan & Kaufmann, 1973). Thus, some magnoliids and *Equisetum* are similar in this respect, although their epidermal pre-patterning differs considerably.

Paracytic stomata also characterize some extinct fossil groups, notably cryptostperms (e.g. *Dicroidium*) and bennettites (Figs 5h,i, 6a–f). In bennettites, although nothing is known about stomatal development, it appears unlikely that their stomata are exclusively perigenous – not because they are paracytic, which is not an indicator of development, but because of the considerable differences in size among the pavement cells and the occasional presence of giant stomata (Table 1). Both of these features constitute potential fossil fingerprints for particular categories of stomatal development.

**VII. Amplifying divisions**

As defined for *Arabidopsis*, an amplifying division (Box 1) is an asymmetric division of an SLGC that creates another SLGC and a meristemoid or GMC (Lau & Bergmann, 2012). In some reticulate-veined taxa, there is a series of one or more amplifying divisions in the GMC lineage, following the initial asymmetric division (Fig. 2d). Amplifying divisions are common in *Arabidopsis*, in which the majority of stomata are surrounded by two or three

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**Fig. 5** Stomata of extinct medullosans (a–c), cordaites (d), cycads (e, f), ginkgophytes (g) and cryptostperms (h, i), from Florin slides in the collection at the Swedish Museum of Natural History. (a–c) *Cyclopteris orbicularis* S010965. (d) Cordaites sp. S20514. (e) *Ptilozamites* sp. S113982. (f) *Ctenis nathorstii* S113983. (g) *Baiera furcata* S113993. (h, i) *Dicroidium feistmantelii* S113986. Bars: (a, e) 100 μm; (b, c, f, h) 20 μm; (d, g, i) 50 μm.
SLGCs, of which one is relatively small (Yang & Sack, 1995; Zhao & Sack, 1999). A similar pattern occurs in many other eudicots (e.g. *Vigna*; Galatis & Mitrakos, 1979) and in some magnoliids (e.g. *Cinnamomum*; Zhao et al., 2006), although not in monocots (e.g. Tomlinson, 1974) or early-divergent angiosperms (Rudall & Knowles, 2013). Amplifying divisions are always asymmetric, so that successive amplifying divisions result in an ‘inward-spiralling’ stomatal complex in which all the cells surrounding the stoma are mesogene cells (Zhao & Sack, 1999; Serna, 2009). As amplifying divisions in *Arabidopsis* are regulated by *SPCH* (e.g. MacAlister et al., 2007), it remains to be seen whether a similar mechanism operates in other, more phylogenetically informative, plants.

The *Arabidopsis* pattern of amplifying divisions occurs in many rosid eudicots (e.g. several other Brassicaceae and several Fabaceae). However, in many other eudicots, only one asymmetric division gives rise to each GMC (e.g. Marx & Sachs, 1977; Payne, 1979). The extent to which pre-patterning influences the presence or absence of amplifying divisions remains undetermined. *Amborella* lacks amplifying divisions and possesses squared pre-patterning (Rudall & Knowles, 2013), but in some other angiosperm taxa, a squared pre-pattern (Box 1) can co-occur with both asymmetric divisions and amplifying divisions, as suggested by images of stomatal development in the eudicot *Pisum sativum* (Kagan et al., 1992).

Among other seed plants, Rudall et al. (2012) reported ‘somewhat chaotic’ amplifying divisions within the stomatal lineage in *Ginkgo*. Maróti (1966) recorded examples of ferns in which the SLGC undergoes one or more divisions into a GMC and one or more mesogene cells. Other examples of successive asymmetric mitoses probably do not constitute amplifying divisions in the strictest sense: in *Equisetum* and some angiosperms, the stomatal precursor cell divides twice asymmetrically to form a paracytic complex (Fig. 2c).

**VIII. Conclusions**

Like many other long-standing questions in biology, the debate surrounding stomatal terminology is not merely semantic but rather is important in framing key questions about the evolution of form. Such questions are ultimately testable using comparative

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**Fig. 6** Stomata of extinct fossil bennettites (a–f), peltasperms (h) and conifers (h–j), from Florin slides in the collection at the Swedish Museum of Natural History. (a, b) *Otozamites bornholmiensis* S113984. (c–f) *Dictyozamites johnstrupii* S113977. (g) *Callipteris conferta* S113987. (h) *Genitzia* sp. S113966. (i, j) *Abietites linkii* S153062. (k) *Androvettia* sp. S153021. (l) *Taxites* sp. S1113961. Bars: (a, c, i) 100 μm; (b, d, e, g, h, j) 20 μm; (f, k, l) 50 μm.
developmental genetics of a selected range of taxa in a phylogenetic context. Perhaps the most important goal in terms of comparative molecular genetics is to determine the roles of orthologues of key developmental genes in phylogenetically pivotal taxa such as Physcomitrella and Selaginella. Payne (1979) hypothesized that the evolution of stomatal patterning is driven by two factors: the structural constraints of guard cells that cannot enlarge relative to neighbouring cells, and a physiological relationship between the guard cells and neighbouring cells, especially in species strongly adapted to resist water loss. Thus, the evolution of diverse stomatal developmental patterns in land plants is potentially linked with environmental factors and climate change. Little is known about possible correlations between stomatal patterning and physiological function (Croxdale, 2000), although it would be interesting to explore potential links between perigenous stomata and physiology relating to Crassulacean Acid Metabolism (CAM), both of which characterize water-lilies. Lateral subsidiary cells could have a physiological role that is subsidiary to that of the guard cells (as reported for grasses; Franks & Farquhar, 2007), but in general it is perhaps more likely that they help to compensate for the contrasts in growth rate between stomata and their neighbours. A holistic approach to leaf development is required to address this question (Payne, 1979; Croxdale, 2000).

Regarding the establishment of ‘fossil fingerprints’ as developmental markers for the regulation of stomatal patterning, we note that it is highly problematic to infer patterns of stomatal development based on the mere absence or presence of subsidiary cells in fossil cuticles (Figs 5, 6). There is no real substitute for examination of fossils showing exceptional anatomical preservation to investigate whether long-short cell alternation occurs in the epidermis of young developing leaves (or the base of linear leaves if development is basipetal). Admittedly, most fossil plants lack such preservation, but other less conclusive indicators of intrinsic asymmetric divisions in the direct stomatal lineage can be sought.

Some potential fingerprints exist in taxa with reticulate venation. For example, a range of variation of epidermal cell sizes and the co-existence of stomata at different developmental stages could suggest that guard-cell formation is preceded by an asymmetric division in the same cell lineage; the presence of ‘giant’ stomata would also suggest this type of development. The existence of a distinct pair of lateral subsidiary cells often results from asymmetric divisions in cells that do not belong to the direct stomatal lineage, a pattern that characterizes grasses and their allies. The occurrence of stomatal islands is typical of taxa with amplifying divisions. Nonrandom stomatal orientation could result either from absence of asymmetric divisions during development (as in water-lilies) or from linear pre-patterning of mesoperigenous stomata, as in monocots, many conifers and probably also bennettites.

The presence of perigenous development in extant bryophytes and lycophytes – groups that evolutionarily preceded all other extant embryophytes (Table 1, Fig. 1) – appears to tentatively support earlier hypotheses that stomatal patterning is ancestrally perigenous in land plants. However, the fundamental importance of division polarity (and hence asymmetric mitosis) in establishing tissue differentiation, especially in unusually physiologically active cells such as stomata and root hairs, suggests that perigenous development could represent a reduction or change in timing of division polarity in mosses and other early land plants. More comparative developmental data are urgently required to address these questions. For flowering plants (angiosperms), the presence of asymmetric divisions in Amborella and Austrobaileyales, and their likely presence in fossil bennettites, suggest that the mesoperigenous condition is ancestral. However, data are needed on stomatal development in putative angiosperm relatives among fossil groups such as bennettites, glossopterids, Pentoxylon and Caytonia (Figs 1, 6). If Caytonia proves to be perigenous, as tentatively indicated by the work of Barbacka & Böka (2000), the ancestral condition in angiosperms is rendered ambivalent during optimization.
The picture is even less clear for seed plants (spermatophytes). Regardless of whether extant gymnosperms are monophyletic, as suggested by most molecular studies (Fig. 1), few observers dispute the stepwise series of extinct Palaeozoic pteridosperm lineages that is reliably placed at the base of the seed plants in morphological analyses (represented in Fig. 1 by Elkinsia, Lyginopteris, Medullosa and Callistophyton). The lack of developmental data for these fossil taxa, and for preceding fossil progymnosperms such as Archaeopteris, obscures the ancestral condition for stomatal patterning in seed plants. Florin (1933) interpreted all of the Palaeozoic pteridosperm lineages as haplochelic (later re-interpreted as anomocytic: Box 1) and perigenous. We concur with other authors (e.g. Pant, 1965; Payne, 1979) in recommending rejection of Florin’s (1933) conceptually hybridogenous terms haplochelic and syndetocheilic in favour of terms that are either purely descriptive (e.g. anisocytic, anomocytic, and paracytic) or (ideally) developmental (e.g. mesogene and peripogene). Carpenter (2005) noted that ‘stephanocytic-like stomatal architecture is found in early seed ferns, as well as fossil cycads, and extant and fossil conifers, some of which bear a strong resemblance to Nymphaeales.’ As stomata of Nymphaeales are perigenous, this type of development could occur in at least some of these other spermatophytes. Rudall & Knowles (2013) tentatively inferred that mesoperigenous stomata are ancestral for angiosperms (Table 1), with an evolutionary loss of asymmetric divisions in Nymphaeales that could reflect a neotenous growth habit promoted by their aquatic environment.

Thus, present data suggest two equally plausible scenarios for patterning the cell lineage leading to guard-cell formation in seed plants: either mesoperigenous development is ancestral or there was an evolutionary loss of asymmetric divisions near the base of the seed-plant clade, followed by one or more subsequent reversals. Asymmetric divisions are not essential for stomatal development, a fact that is demonstrated by the presence of both perigenous and mesoperigenous development in both Amborella (Rudall & Knowles, 2013) and Ginkgo (Rudall et al., 2012), the latter account contradicting earlier reports of exclusively perigenous development in Ginkgo. Fuelled by plant hormones, there exists a dynamic relationship between the plant apical meristem, which lays down the basic tissue, and the more distal formation of meristemoids, which further differentiate the tissues to form cells such as stomata and root hairs that together provide a key environmental interface. Given that meristemoid differentiation in Sphagnum occurs only when the apical cell has ceased to divide (Bünning, 1952), an interesting future question is to determine whether evolutionary changes in stomatal meristemoid development and patterning are related to holistic aspects of growth forms in more derived groups of land plants.

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