REVIEW

A Re-examination of the Root Cortex in Wetland Flowering Plants With Respect to Aerenchyma

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● *Aims* We review literature and present new observations on the differences among three general patterns of aerenchyma origin and their systematic distributions among the flowering plants, and we clarify terminology on root aerenchyma.

● *Scope* From our own previous works and some new observations, we have analysed the root cortex in 85 species of 41 families in 21 orders of flowering plants that typically grow in wetlands to determine the characteristic patterns of aerenchyma.

● *Findings* A developmental and structural pattern that we term expansigeny, as manifested by honeycomb aerenchyma, is characteristic of all aquatic basal angiosperms (the Nymphaeales) and basal monocots (the Acorales). Expansigenous aerenchyma develops by expansion of intercellular spaces into lacunae by cell division and cell expansion. Schizogeny and lysigeny, so often characterized in recent reviews as the only patterns of root cortex lacunar formation, are present in most wetland plants, but are clearly not present in the most basal flowering plants.

● *Conclusion* We conclude that expansigeny is the basic type of aerenchyma development in roots of flowering plants and that the presence of expansigenous honeycomb aerenchyma in root cortices was fundamental to the success of the earliest flowering plants found in wetland environments.

Key words: Aerenchyma, air spaces, angiosperms, cortex, expansigeny, flowering plants, honeycomb aerenchyma, lysigeny, roots, schizogeny.

INTRODUCTION

Our goal in this review is to update our present state of knowledge on the development, structure and systematic distribution of root cortex tissues that are fundamental to adaptations of wetland plants to their normal habitats. The root cortex of angiosperms is a primary tissue derived from cortical initials in the root apical meristem (hereafter referred to as the RAM comprising the stele, cortex, epidermis and rootcap). Its intercellular spaces and parenchyma cells between the endodermis and hypodermis can be modified to create enlarged air chambers, cavities or spaces. By reviewing the historical record and by using our previous works and plants about which we have not previously published, we shall demonstrate that development of a honeycomb pattern of aerenchyma in root cortex is basic to flowering plants and is fundamentally different from schizogeny and lysigeny.

Early reports

The notion that the root cortex of flowering plants that grow in aquatic or wetland environments under hypoxic conditions is comprised primarily of enlarged air spaces, or lacunae, has been considered for at least 150 years. Schleiden's (1849, p. 54) very brief description of air canals and air cavities represents one of the earliest scientific depictions of air canals in the literature; Schleiden noted that such 'receptacles of air' are 'formed by the destruction of a mass of parenchyma'. Sachs (1872, p. 73) noted that separations (splitting) between cells during development or deaths of cells led to expanded air spaces. Shortly thereafter, De Bary (1877, p. 204–205), depicted the 'schizogenetic' and 'lysigenetic' processes by which enlarged air chambers are produced; this appears to have been the first use of the words and definitions for schizogeny and lysigeny. De Bary (1877) also used other expressions, such as rhexigenetic (mechanical rupture). De Bary characterized air chamber production in *Jussiaea* (*Ludwigia*) to describe the air spaces found in that genus. Interestingly, his description of the pattern of development did not fit into either of his major categories of schizogenetic or lysigenetic development.

Aërenchym/aerenchyma

Tissue with enlarged spaces was originally defined by Schenck (1890, p. 526) as 'Aërenchym', or aerenchyma in English. Schenck used peridermal tissue to define 'Aërenchym', but he also described aerenchyma in cortex tissue. We will not address any air spaces derived from phellogen (cork cambium) as peridermal or secondary aerenchyma although it is very important for some plants, especially woody plants with secondary growth of roots (see Stevens *et al.*, 2002).

After Schenck (1890) reported on aerenchyma in members of the Onagraceae (including *Jussiaea*), Lythraceae and Melastomaceae, Goebel (1891) suggested a broader scope for Schenck's term so that it included primary tissues, not just periderm. Further, Haberlandt (1896) utilized the concepts of schizogeny and lysigeny to describe in more depth how air spaces arose and functioned in gaseous exchanges throughout plants. In his use of these concepts, Haberlandt (1896) specifically summarized Schenck's (1890) study and reproduced Schenck's drawings to depict 'Aërenchym' origin for primary tissues.

Importance of Schenck, Conard and Němec

Schenck (1890), most importantly, did not use any terms that could be translated as schizogeny or lysigeny. Indeed, he specifically described differential elongation of parenchyma cells in the central cortex to produce the enlarged air chambers and aerenchyma. Following Schenck, two other authors presented salient works that have been all but ignored. Conard (1905) reported patterns of air space expansion into air canals in the genus *Nymphaea*; in his description, Conard avoided use of the terms schizogeny and lysigeny, instead referring to cell expansions and divisions resulting in air space expansion into 'hexagonal canals' from intercellular spaces, which themselves were first formed in the RAM at the tip of the cortex.

Němec (1907, 1930) then explained the processes of aerenchyma formation found in both the Nymphaeales and Acorales. After initially stating in his first book that schizogeny and lysigeny produced air chambers, Němec (1907, p. 382) explained that cell division and cell expansion resulted in the enlargement of air spaces into lacunae, as follows: 'Schizogenous and lysigenous spaces could enlarge due to growth of cell walls and division of cells which surround the space, which is typical especially for intercellular spaces of water plants. . . .' (translation by O.V.).

Němec (1907, p. 388) noted: 'In sweet flag (*Acorus calamus*) roots, longitudinally oriented air channels are formed which reach very close to the tip. These channels do not merge radially, they look like circles on the cross section and every channel is surrounded by the wall which looks like a wreath of cells. The channels originate as small spaces along the cell corners and are enlarged due to growth of surrounding cells. Simultaneously, the cells around the spaces divide, the new walls being oriented perpendicularly to the channels' (translation by O.V.). Contrary to his own initial statement, Němec did not demonstrate any cell separations and deaths leading to the lacunae; instead, he clearly attributed the phenomena of cell divisions and expansions to the enlargement of intercellular spaces into lacunae, as he also demonstrated in his Fig. 212A and 212B (pp. 382 and 383, respectively). Clearly, however, Němec reflected the attitude of his era with his use of the terms schizogeny and lysigeny because they were already a matter of convention for plant anatomists—except for Schenck, even though the actual processes of space expansion were, in fact, not schizogenous or lysigenous. Years later, Němec (1930) depicted the same pattern of aerenchyma development

and structure in *Nuphar luteum* of the Nymphaeaceae as he had reported for *Acorus* in 1907 and as Conard had shown for *Nymphaea* in 1905; here, Němec did not use the terms schizogeny or lysigeny.

The salience of these reports is that there had been established a different set of processes for development of root cortical aerenchyma. The patterns of aerenchyma development found in two major plant orders, the Nymphaeales and Acorales, now considered basal angiosperms and basal monocots, respectively (APG 2003; Soltis and Soltis, 2004), were found not to be schizogenous or lysigenous, but expansigenous, i.e. growth of intercellular spaces into lacunae by cell division and enlargement. Yet, the use of the terms schizogenous and lysigenous had clearly become convention in spite of the reports in the literature and Schenck's definition.

As we examined other early 20th Century scholarly treatises, we found that nearly all other scholars continued, as if by convention, to refer to air space expansion as either schizogenous or lysigenous. In her broad approach to the study of wetland plants, Arber (1920) depicted many features of aquatic/wetland plants, including their roots, but she did not cite the reports of Conard or Němec, in spite of her extensive annotated bibliography. The early to mid-20th Century period can be summarized best by Sifton's reviews (1945, 1957), Yamasaki's (1952) study, a paper by Hasman and İnanç (1957), one of the Pillai's papers (Pillai and Pillai, 1962) and Sculthorpe's well known treatise (1967); schizogeny and lysigeny were depicted as the only patterns of aerenchyma origin.

Schizogeny/lysigeny and recent reviews

While recent reviews (see below) have emphasized lysigeny and schizogeny, we have found relatively few detailed studies after the early reports (De Bary, 1877; Haberlandt, 1896) from the 1800s. Němec (1907) noted schizogeny in *Rorippa* and schizo-lysigeny ('schizolysigenní', p. 387), and Stover (1928) reported schizogeny in *Echinochloa*. More recent studies depicting examples of schizogeny and schizo-lysigeny included a broad study by Justin and Armstrong (1987) and a detailed study on *Typha* by Seago and Marsh (1989).

Stover (1928) depicted lysigeny in *Zizania aquatica*, a wetland grass. Boeke (1940) presented what may have been the most detailed and careful explanation of the origin of 'intercellulary cavities' in rice that he determined to be schizogenous; he even accounted for sectioning difficulties resulting in tears of tissue that made the tissues seem lysigenous. In the Hydrocharitaceae, Tomlinson (1969) analysed lysigeny and diaphragm formation in *Thalassia*, and Seago *et al.* (1999*a*) described an unusual pattern of lysigeny in *Hydrocharis morsus-ranae*. Other recent researchers, e.g. Gaynard and Armstrong (1987), Clark and Harris (1981), Webb and Jackson (1986), Kawai *et al.* (1998) and Soukup *et al.* (2002), have illustrated or described details of lysigeny in Cyperaceae and Poaceae (especially rice), although, in each case, an examination of the micrographs demonstrates that schizogeny always precedes or accompanies lysigenous space formation.

Another unusual pattern of cell space expansion in *Sagittaria* was noted first by De Bary (1877), later redescribed by Severin (1932) and Schussler and Longstreth (1996), who characterized it as lysigenous, before Longstreth and Borkhsenious (2000) refined the characterization of its aerenchyma to schizogeny with collapsed, but not dead cells. In fact, Longstreth and Borkhsenious (2000) have called into question whether or not root aerenchyma which appears lysigenous really contains dead cells; they hypothesize that many of the cells in some plants are just collapsed.

While there have been experimental studies on wetland plants since at least Haberlandt (1896), in the later 20th Century, Armstrong (e.g. 1979) and others began in-depth experimental studies to elucidate the functions of aerenchyma after the Williams and Barber (1961, p. 132) classic hypothesis on the 'functional significance of aerenchyma'. A major study on categories of air spaces in roots of flowering plants by Justin and Armstrong (1987) determined cell packing arrangements that enabled air spaces to form and linked cell packing to root porosity; they did not study any Nymphaeales.

The 25 years following Armstrong's major review are summarized by recent reviews by Blom (1999), Jackson and Armstrong (1999), Armstrong and Drew (2002) and Evans (2004). However, an emphasis on understanding the mechanisms behind cell separations and cell deaths has been placed on non-wetland plants that are induced to form air spaces, as illustrated by the research on maize characterizing programmed cell death in lysigenous aerenchyma formation and roles of plant growth regulators and enzymes (e.g. Gunawardena *et al.*, 2001*a*, *b*). A recent review on cell separation processes in plants very briefly mentioned the mechanisms causing schizogenous aerenchyma in roots (Roberts *et al.*, 2000). Also, the flow of gases through the organs and tissues of wetland plants growing in anoxic or anaerobic environments has been much studied by researchers such as Armstrong *et al.* (1999).

These suffice to illustrate the varying analyses of the developmental, physiological and ecological aspects of aerenchyma in flowering plants, including their roots. Most of the researchers have also described and defined, to varying degrees, the development and structure of aerenchyma (see Justin and Armstrong, 1987), with an emphasis on the relationship to functional aspects. Their definitions can be summarized by some simple statements in Evans (2004, Fig. 1, p. 2) that there are only two patterns of aerenchyma origin: 'schizogeny' or 'separation of cells', and 'lysigeny' or 'death of some cells'.

Another pattern of aerenchyma

In spite of the foregoing and in general agreement with Schenck in 1890, first Conard (1905), then Němec (1907, 1930), Ellmore (1981), Seago *et al.* (2000*a*, *b*) and Seago (2002) presented detailed descriptions of root aerenchyma development and structure that are at variance with the well-known types. Seago *et al.* (2000*a*, *b*) applied the expression 'differential expansion' to the two different patterns they detected in *Nymphaea* and *Pontederia*; the 'differential expansion' found in *Pontederia* (Seago *et al.*, 2000*b*) is similar to that found in *Ludwigia* (Ellmore, 1981), i.e. Schenck's *Jussiaea*. Longstreth and Borkhsenious (2000) and Armstrong and Drew (2002) noted that the depiction by Seago *et al.* (2000*a*, *b*) might represent another pattern of aerenchyma origin that departs from the schizogeny–lysigeny scenarios, but they did not emphasize its significance.

We suggest that the concepts of schizogeny and lysigeny, used to describe the development of aerenchyma tissue in the root cortex of flowering plants (see Justin and Armstrong, 1987; Jackson and Armstrong, 1999; Armstrong and Drew, 2002; Evans, 2004), are inadequate to account for the different methods of origin of this primary tissue in the roots of the angiosperms.

The basal angiosperm aerenchyma

Accordingly, we propose that aerenchyma in which a combination of cell division and cell enlargement leads to an expansion of intercellular spaces into lacunar aerenchyma is the basic pattern of aerenchyma origin and structure in the early flowering plants, and we present evidence for its different manifestations. Our emphasis in this article will be on this pattern of aerenchyma, which we term expansigeny (or honeycomb aerenchyma; Laan *et al.*, 1987), because it has been so overlooked and because schizogeny and lysigeny have been treated so extensively. We shall not report on the association between aerenchyma and the two barrier regions of the cortex, endodermis and hypodermis (including exodermis).

PLANTS EXAMINED AND TERMINOLOGY

Plants examined

We examined 85 species from 41 families in 21 orders of flowering plants (Table 1; the unplaced family Boraginaceae is treated as the order Boraginales herein). Our previous studies have presented the methods by which we have collected specimens and analysed root tissues (Seago and Marsh, 1989; Seago *et al.*, 1999*a*, *b*, 2000*a*, *b*; Seago, 2002; Soukup *et al.*, 2002; Stevens, 2003). However, we also collected additional specimens from growers, aquatic gardens or wetlands in which the roots of plants were growing in wetland conditions—from total submersion in open water to soils flooded up to the transition from roots to stems or points of origin of adventitious roots. The roots we examined were mostly adventitious roots because adventitious roots dominate wetland plants and many plants simply do not produce primary roots upon seed germination [see, for example, Schenck, 2003 (1886); Arber, 1920]. We often examined many root specimens (>50) from multiple plants of a species, but we also had to examine only a few roots from a single plant for a few species because plants were not available; we were sometimes limited by appropriate restrictions against our harvesting roots obtained from aquatic or wetland plants that grew in botanical gardens or other institutions. Root specimens were harvested, treated and examined under brightfield or UV epifluorescence
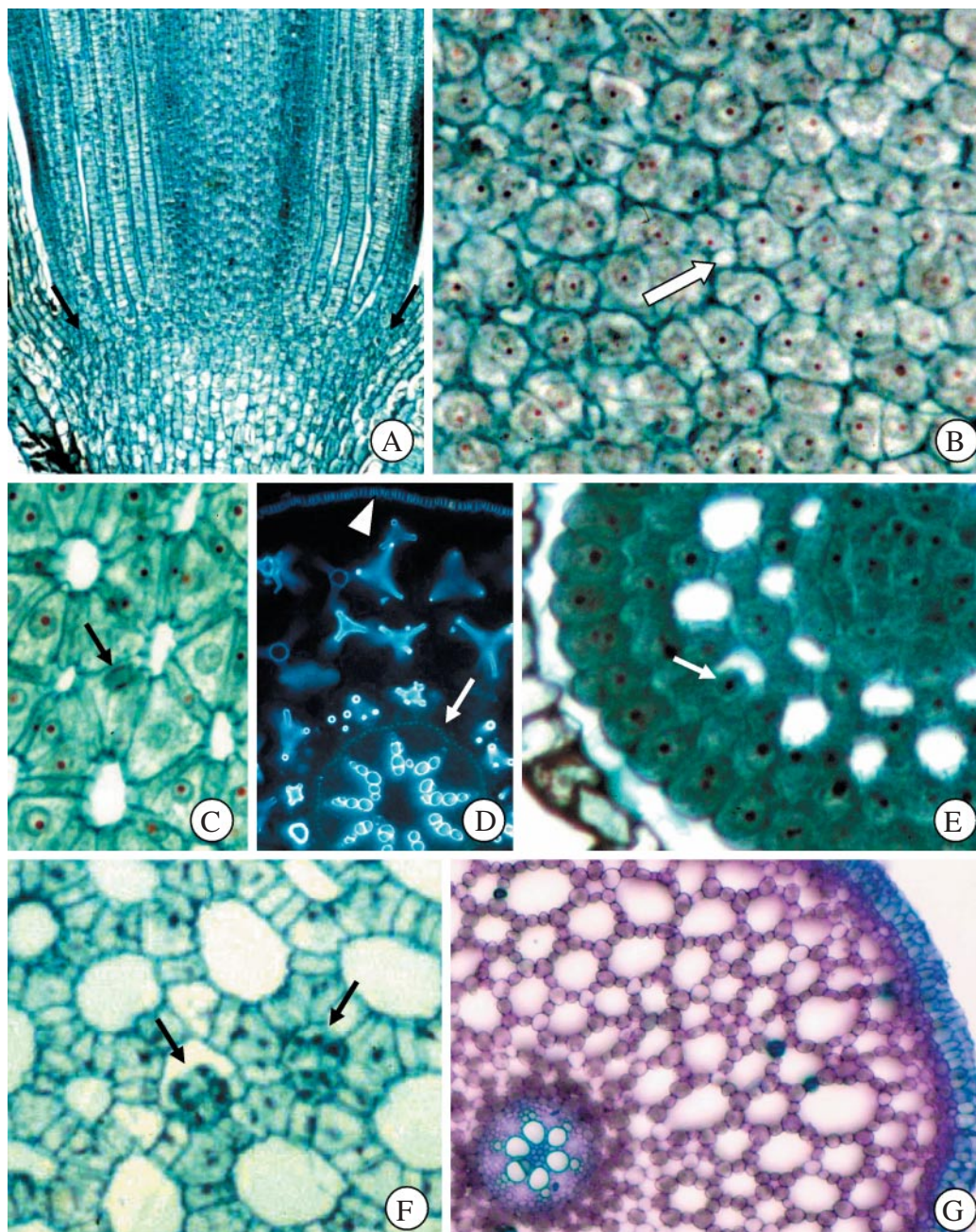
FIG. 1. Origin of honeycomb aerenchyma or expansigeny in roots of Nymphaeales and Acorales. Roots are 10–30 cm long, except *Cabomba*, which was 8–9 cm. (A) *Nymphaea odorata*. Longisection of an open RAM. The cleft above the arrow shaft on the left separates the rootcap from the epidermis. The RAM is between the arrows. (B) *Nymphaea odorata*. Section at the proximal end of cortical initials within RAM. Note the irregular arrangement of cells. The arrow shows the intercellular space at cortical initials. (C) *Nymphaea odorata*. Section 200 μm proximal to RAM. The arrow points to a mitotic figure cleaving off a narrow cell from a semi-regular hexagon cell. (D). *Nymphaea odorata*. Section with fluorescing astrosclereids in cortex between endodermis (arrow) and exodermis (arrowhead) from mature root. Reproduced with permission of the *Journal of the Torrey Botanical Society*. (E) *Cabomba caroliniana*. Section at 300 μm behind RAM with an initial diaphragm cell protruding into a lacuna shown by the arrow. (F) *Nymphaea odorata*. Section at 1000 μm behind RAM with stages of multiseriate, transverse diaphragms shown by the arrows. (G) *Acorus calamus*. Transverse section near the base of a mature adventitious root with honeycomb expansigenous aerenchyma.

microscopy (only UV epifluorescence is noted in the figure legends), as we have described previously (Seago and Marsh, 1989; Seago *et al*., 1999*a*, 2000*b*; Soukup *et al*., 2002; Stevens, 2003). Root apical sections were not examined for all species.

Terminology

We decided to use the term expansigeny for the following reasons. The cortical anatomies described below have been presented previously, but researchers either used only the word expansion (Ellmore, 1981) or the expression

TABLE 1. *Wetland plants studied (sequence after Angiosperm Phylogeny Group, 2003)*

Basal angiosperms
  Nymphaeales
    Nymphaeaceae—*Nymphaea odorata, Nuphar luteum, Victoria amazonica*
    Cabombaceae—*Brasenia schreberi, Cabomba caroliniana*
Monocots
  Acorales
    Acoraceae—*Acorus calamus*
  Alismatales
    Araceae—*Calla palustris, Pistia stratiotes*
    Hydrocharitaceae—*Stratiotes aloides, Hydrocharis morsus-ranae, Vallisneria americana, Najas quadalupensis*
    Butomaceae—*Butomus umbellatus*
    Alismataceae—*Sagittaria latifolia, Baldellia ranunculoides*
    Aponogetonaceae—*Aponogeton ulvaceus*
    Potamogetonaceae—*Potamogeton nodosus* and *pectinatus*
  Asparagales
    Orchidaceae—*Habenaria sp.*
    Iridaceae—*Iris pseudacorus, versicolor* and *virginica*
    Amaryllidaceae—*Leucojum aestivum*
  Poales
    Sparganiaceae—*Sparganium* sp.
    Typhaceae—*Typha glauca* and *angustifolia*
    Juncaceae—*Juncus effusus & glaucus*
    Cyperaceae—*Cyperus alternifolius, Scirpus zebrinus* and *lacustris, Eleocharis* sp., *Carex gracilis, Cladium jamaicense*
    Poaceae—*Glyceria grandis, maxima, & striata, Molinia coerulea, Phragmites australis, Spartina alterniflora, Oryza sativa*
  Commelinales
    Pontederiaceae—*Pontederia cordata, Eichhornia crassipes*
  Zingiberales
    Cannaceae—*Canna* sp. 'Pretoria'
Eudicots
  Ranunculales
    Ranunculaceae—*Caltha palustris, Ranunculus repens*
  Proteales
    Nelumbonaceae—*Nelumbo lutea*
  Gunnerales
    Gunneraceae—*Gunnera manicata*
  Caryophyllales
    Droseraceae—*Drosera adelae* and *capensis, Dionaea muscipulo*
    Polygonaceae—*Rumex crispus*
  Saxifragales
    Haloragaceae—*Myriophyllum aquaticum*
    Crassulaceae—*Crassula aquatica*
  Myrtales
    Lythraceae—*Decodon verticillatum, Lythrum salicaria, alatum* and *hysoppifolia, Trapa natans*
    Onograceae—*Epilobium hirsutum, ciliatum, parviflorum* and *angustifolium, Ludwigia repens*
  Malpighiales
    Elatinaceae—*Elatine hexandra*
    Euphorbiaceae—*Euphorbia palustris*
  Fabales
    Fabaceae—*Neptunia prostrata, Lathyrus pratensis*
  Brassicales
    Brassicaceae—*Rorippa officinale*
  Ericales
    Balsaminaceae—*Impatiens capensis, pallida* and *glandulifera*
    Sarraceniaceae—*Sarracenia rubra*
  Boraginales (Euasterid I—unplaced family)
    Boraginaceae—*Myosotis scorpoides*
  Lamiales
    Acanthaceae—*Justicia americana, Hygrophila difformis*
    Scrophulariaceae—*Mimulus ringens*
    Lentibulariaceae—*Pinguicula bohemica*
  Apiales
    Apiaceae—*Hydrocotyle verticillata*
  Asterales
    Menyanthaceae—*Nymphoides cristata, crenata, peltata* and *indica, Menyanthes trifoliata*
    Asteraceae—*Bidens tripartitus*

differential expansion (Seago *et al.*, 2000*a, b*). We wanted to find a word or expression for this phenomenon that would be comparable with schizogeny and lysigeny because we presume that the first use of the terms (see De Bary, 1877) came from their Latin transliterations of Greek. We did not find any comparable Latin transliteration from Greek for words for expansion, as there are words for schizogeny and lysigeny; so we settled upon the term, expansigeny, which derives from the Latin verb, expando, meaning 'to stretch out, expand, spread out' from *Cassell's Latin and English Dictionary* (Simpson, 1987, p. 85), and the Greek for origin, -geny, for two important reasons. First, both the spaces/lacunae and the cells surrounding them can be described as expanding or undergoing expansion during development of aerenchymatous lacunae. Secondly, the proteins involved in extension growth of cell walls, expansions (see Cosgrove, 1999, 2000), are obviously relevant to the expansion growth of cells around lacunae. It is logical to refer to the pattern of cortical lacunar and cell enlargement in a manner which reflects their expansion, i.e. cells expand differentially by cell division and by extension of walls lining the enlarging spaces with concomitant space expansion. Thus, we adopt the word expansigeny, origin by expansion, without further cell separations (schizogeny) or cell deaths (lysigeny).

FINDINGS

In the roots of plants that are normally growing under wetland conditions, we characterize the development and organization of cortical lacunae, i.e. air chambers or cavities, into aerenchyma or as intercellular spaces as follows.

Expansigeny

The expansion of intercellular spaces into lacunae, or air chambers, by cell division and cell enlargement, without further cell separations and without cell collapse or death, is termed expansigeny. In roots of plants such as *Nymphaea*, intercellular spaces are found within the RAM cortical initials which, with the epidermal initials, form an open meristem across the tip of the cortex (Fig. 1A; Clowes, 2000; Seago *et al.*, 2000*b*); these small RAM intercellular spaces arise by schizogeny and can be seen in a variety of plants (e.g. see Soukup *et al.*, 2002). Intercellular spaces grow into lacunae by cell divisions and cell expansions of the initial 4–6 cells around each space (Fig. 1B and C); unequal cell divisions result in enlarged cells, termed semi-regular hexagons (Seago *et al.*, 2000*b*), and narrow cells (Fig. 1C). Cell divisions can then occur in both types of cells. There are no further cell separations or cell collapses, although in the Nymphaeaceae the semi-regular hexagon cells produce astrosclereids (Fig. 1D; Conard, 1905; Seago *et al.*, 2000*b*). In the Cabombaceae and Nymphaeaceae, uniseriate, transverse diaphragms (unicellular to bicellular, Fig. 1E, or multicellular, Fig. 1F, respectively) are produced that separate scattered lacunae into longitudinal chambers (see Conard, 1905; Harada, 1979; Seago *et al.*, 2000*b*). This type of expansigeny, which is characterized by a hexagonal, polygonal or honeycomb appearance in transverse section (Conard, 1905; Laan *et al.*, 1987), is termed honeycomb
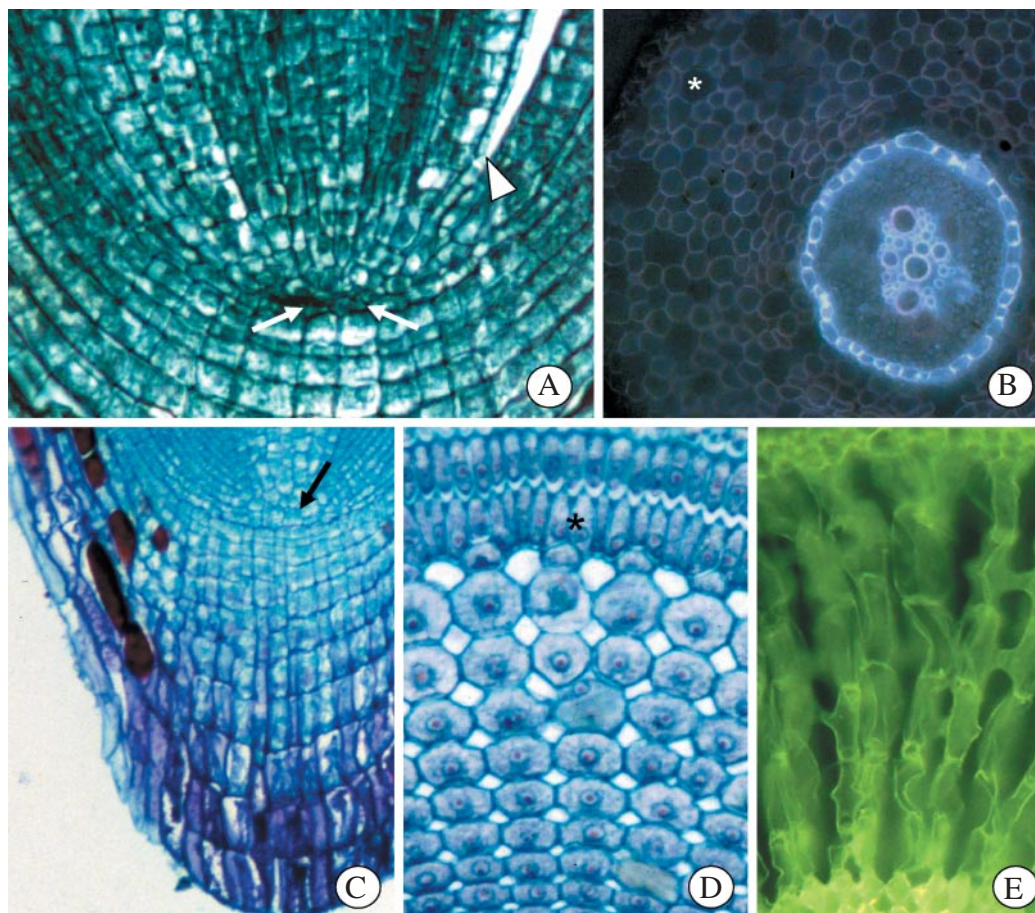
FIG. 2. Honeycomb and radial expansigeny in roots. (A) *Hydrocotyle verticillata*. Median longitudinal section of a closed RAM (arrows show the cortical initials; epidermal initials associated with root cap initials) and the tip of distal-most, newly initiated lacuna near RAM in the upper right above the arrowhead. The root is 10 cm long. (B) *Rumex crispus*. Epifluorescent image showing expansigeny in the cortex near the base of a 10 cm long adventitious root, just after secondary growth had started; the asterisk indicates a lacuna. (C) *Pontederia cordata*. Median longitudinal section of RAM with closed, one-tiered cortical–epidermal initials shown by the arrow in a 25 cm long root. (D) *Pontederia cordata*. Section at 200 μm behind RAM of a 20 cm root with slightly enlarged intercellular spaces and cortical cells, especially in the outer cortex; the prohypodermis is shown by an asterisk. (E) *Pontederia cordata*. Section through the base of a 16 cm root illustrating radial expansigeny in aerenchyma of central cortex under epifluorescence. Reproduced with permission of the *American Journal of Botany*.

expansigeny or honeycomb aerenchyma. In the monocots, *Acorus calamus* (Acorales, Acoraceae; Fig. 1G), *Najas* (Alismatales, Hydrocharitaceae) and *Habenaria* (Asparagales, Orchidaceae) also form honeycomb aerenchyma, but lack diaphragms. *Acorus*, for example, forms ground meristem with irregular or non-radially aligned files or layers of cells from the proendodermis to the hypodermis that is similar to the Nymphaeales.

Expansigenous aerenchyma can also occur in eudicots that have roots with open meristems such as *Nelumbo luteum* or plants that have closed RAMs with one or two tiers of cortical initials (Fig. 2A; epidermal initials associated with rootcap initials), as in *Rumex* and *Hydrocotyle*. In these roots, oblique divisions occur first in outer portions of ground meristem cells of a file and produce derivatives that increase the number of cells around the intercellular spaces. Subsequent enlargement of the cells results in lacunae. We have not found diaphragms or sclereids in the diverse, expansigenous eudicots (Fig. 2B).

Radial expansigeny is characteristic of the Pontederiaceae (*Eichhornia* and *Pontederia*; see Seago *et al*., 2000*a*). In these plants and in some members of the Onagraceae (Schenck, 1890; Ellmore, 1981; Longstreth and Borkhsenious, 2000), a closed RAM of one tier of cortical–epidermal initials (Fig. 2C; the rootcap has a separate tier of initials) produces a ground meristem, strongly radiating out from the proendodermis in radial files of cells (Fig. 2D). Then, differential cell expansion along cell walls, starting in the walls lining the intercellular spaces in the outer cortex near the hypodermis (Fig. 2D), is followed by radial and some limited tangential wall expansion throughout the mid-outer cortex that results in expanded intercellular spaces in the mature root (Fig. 2E).

Schizogeny

The expansion of intercellular spaces into lacunae along radial sectors between radially adjacent cells or sectors of
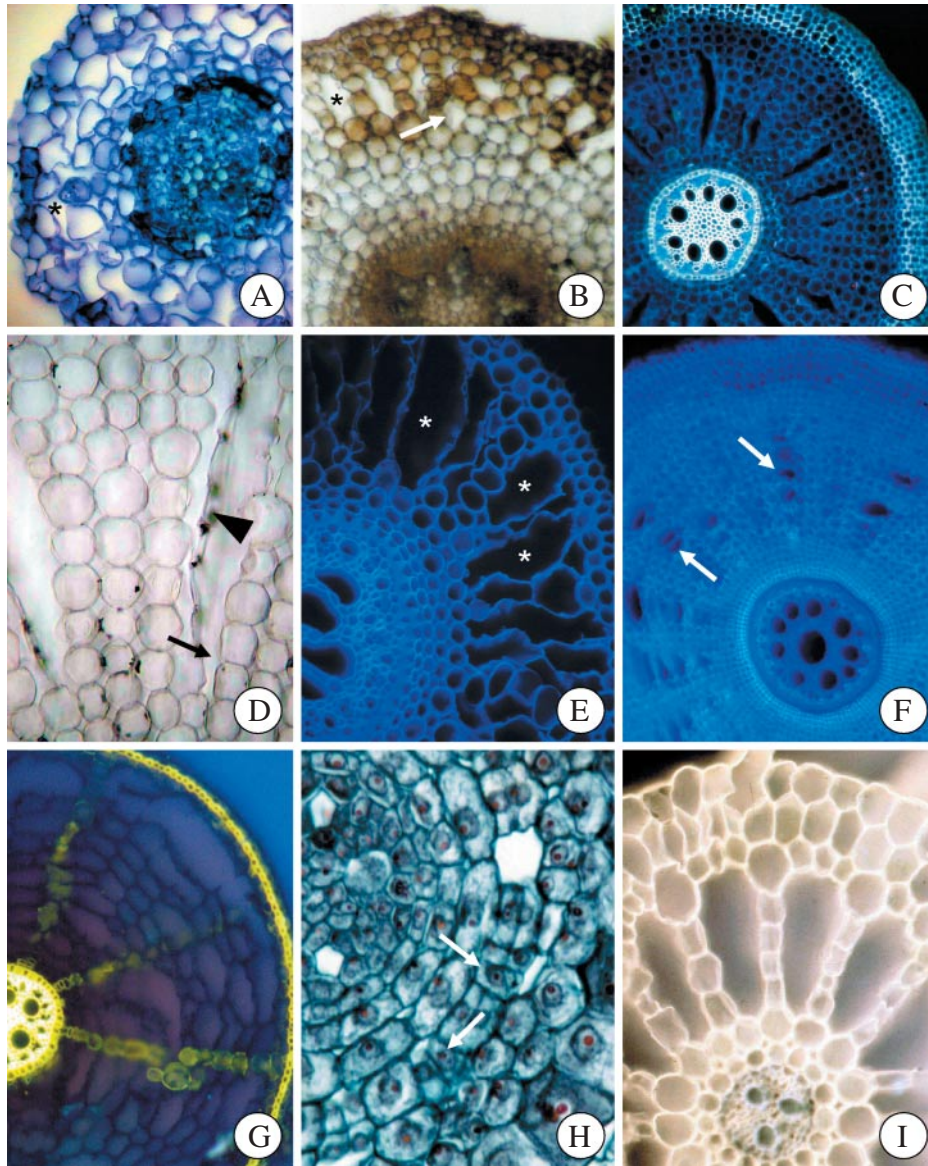
FIG. 3. Schizogeny, schizo-lysigeny, mixed aerenchyma and lysigeny. (A) *Epilobium parviflorum*. Section of a long mature root with schizogenous aerenchyma in the cortex; note the lacuna indicated by the asterisk. (B) *Neptunia prostrata*. Section of a mature 15–20 cm root with mixed aerenchyma: some schizogenous lacunae (asterisk) and some expansigenous lacunae (arrow). (C) *Typha glauca*. Section of a mature 25 cm root with schizo-lysigenous aerenchyma shown under epifluorescence. (D) *Phragmites australis*. Section 300 μm behind the RAM of a 10 cm root, illustrating early stages of aerenchyma formation; the arrow indicates the schizogenous space, and the arrowhead shows lysigeny. (E) *Glyceria maxima*. Epifluorescence of the base of a mature root with radial lysigenous spaces indicated by the asterisks. (F) *Cyperus alternifolius*. Section through a ground meristem at 25 mm behind cortical initials showing the beginnings of the schizogenous and lysigenous origin of lacunae; the 30 cm long root is shown under epifluorescence. The arrows indicate early tangential lysigenies. (G) *Cladium jamaicense*. Section through the base of a mature 20 cm long adventitious root with tangential lysigeny shown under epifluorescence. (H) *Hydrocharis morsus-ranae*. Section at 300 μm behind cortical initials of a 12 cm aquatic root; arrows indicate the redivided ground meristem cells. (I) *Hydrocharis morsus-ranae*. Epifluorescent image near the base of a mature 12 cm aquatic root illustrating packet lysigeny. Reproduced with permission of the *Canadian Journal of Botany* and NRC of Canada.

cells by the separation of cells along their common walls so that radially or obliquely aligned intercellular spaces join is termed schizogeny (Fig. 3A); these kinds of cortices are almost always derived from closed RAMs with one tier of cortical initials, similar to Fig. 2A. It appears that many species have roots with schizogenous lacunae that are irregular or somewhat scattered in distribution throughout the cortex (some roots of the Fabaceae), but only some species (e.g. *Rorippa officinale*, Brassicaceae, Brassicales; see also Němec, 1907) are characterizd by having wide-spread schizogeny. Some roots of plants such as *Typha* (Typhaceae, Poales) have schizogenous aerenchyma in regions of the cortex near the bases of many roots (Seago and Marsh, 1989; Seago *et al.*, 1999*b*; see below).
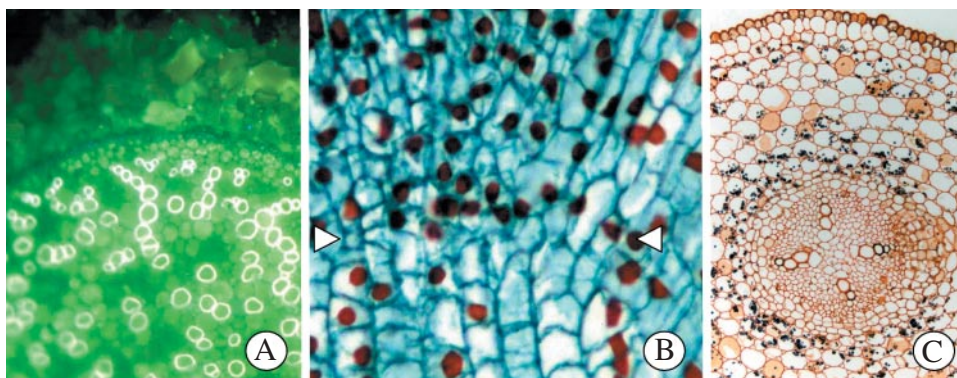
Fɪɢ. 4. Roots with no aerenchyma. (A) *Drosera capensis*. Section through the base of an old 9–10 cm root; epifluorescent image. Cortex can be seen across the top. (B) *Gunnera manicata*. Median longitudinal section of open RAM in a new, 9 cm long, adventitious root grown in flooded soil; arrowheads delimit RAM. (C) *Euphorbia aquatica*. Section through the base of an old root. No aerenchyma.

Mixed aerenchyma

Combinations of schizogeny and some expansigenous lacunae, or mixed aerenchyma, can be found in the cortex of some species in the Fabaceae such as *Neptunia* (Fig. 3B) in which a mixture of mostly schizogenous lacunae and some limited expansigenous lacunae occurs. This may be related to the kinds of RAMs present in the Fabaceae; they always have RAMs with open meristems in which the epidermis is associated with the rootcap (see below), but the initials for the cortex have the general configuration as the cortical initials in the Nymphaeaceae (Clowes, 2000; J. L. Seago, Jr, unpubl. res.). It is, however, very difficult to determine in some cases whether or not lacunae were produced by cell divisions/expansions or by separations between cells, and some lacunae probably arise by separations of cells and cell expansions.

Schizo-lysigeny

More commonly for *Typha*, the separation of cells along radial walls is followed by deaths of some cells, especially cells which produce crystals and then break apart; this is termed schizo-lysigeny (Fig. 3C; see also Němec, 1907). In plants such as *Typha*, the second wave of adventitious roots during a growing season contains much schizo-lysigenous aerenchyma (see Seago and Marsh, 1989; Seago *et al.*, 1999*b*).

Lysigeny

The collapse and death of cells to create lacunae, coupled with cell separations that usually precede cell collapse, is lysigeny. At least three distinct patterns of lysigenous origin of lacunae can be identified: radial, tangential and packet. *Phragmites* and *Glyceria* represent good examples of the radial lysigeny pattern in which schizogenous separations are accompanied or followed by collapse and death of cells along radial sectors of the mid cortex (Fig. 3D, E; Soukup *et al.*, 2002). The aerenchyma in the Cyperaceae arises by tangential lysigeny; cell separations and cell collapses occur in tangential sectors between intact radial files of cells (Fig. 3F, G). In *Hydrocharis* and *Stratiotes* of the Hydrocharitaceae, cells in radial files divide anticlinally, but somewhat unequally, to produce extra cells in a file, and then all or most of the newly divided cells collapse and die to produce lacunae (Fig. 3H, I; Seago *et al.*, 1999*a*). Such packet lysigeny is also found in *Pistia stratiotes* of the Araceae (Alismatales).

In all of these lysigenous aerenchymas in monocots, the RAMs of the plant roots are closed with one tier of cortical–epidermal initials (see Fig. 2C), except in the Hydrocharitaceae where there are often separate tiers of initials for the cortex and epidermis, giving rise to a four-tiered RAM (see Seago *et al.*, 1999*a*). In eudicots with radial lysigeny, the RAMs are basically closed, with one tier, or sometimes two tiers, of cortical initials, but the epidermal initials are associated with the rootcap (as in Fig. 2A).

No aerenchyma/intercellular spaces

The continued enlargement of the initial, tiny intercellular spaces into slightly enlarged intercellular spaces leaves a condition in which there is no aerenchyma, i.e. no lacunar cortex; there may, of course, be a very few schizogenous or lysigenous spaces as roots age. In the materials we examined, the Gunnerales, some species of the Saxifragales, Malpighiales, Asterales, some roots of members of the Fabaceae, and carnivorous plants growing in bog conditions, e.g. Droseraceae (Caryophyllales; Fig. 4A), Sarraceniaceae (Ericales) and Lentibulariaceae (Ericales), were not observed to form lacunae; their intercellular spaces exhibit only a slight enlargement. Plants such as *Gunnera manicata* (Gunnerales, Gunneraceae) have open RAMs (Fig. 4B) with the epidermis arising in association with the rootcap as in the Fabaceae, and the ground meristem is not comprised of radially aligned cell files. *Euphorbia aquatica* is an example of a species with no aerenchyma (Fig. 4C).

Another situation is found in plants such as *Typha* that produce roots in waves of adventitious root production (see Seago and Marsh, 1989; Seago, *et al.*, 1999*b*). The earliest roots produced in a growing season often have no aerenchyma, in spite of being immersed in the saturated substrate, while subsequent waves of adventitious roots are often

characterized by schizogenous and schizo-lysigenous aerenchyma.

Other features found in aerenchyma

Crystalliferous cells may be present; when they break down, as in *Typha* (Seago and Marsh, 1989), schizo-lysigenous lacunae and aerenchyma are formed. Intact crystalliferous cells can be found in such diverse plants as *Pontederia* (Commelinales; Seago *et al.*, 2000*a*) or *P. stratiotes* (Araceae, Alismatales).

Sclereids, especially astrosclereids, are common in the Nymphaeaceae (Fig. 1D) of the basal angiosperms and in the Menyanthaceae (*Nymphoides*) of the most advanced Eudicots (Seago and Seago, 2004). While their close relative, *Menyanthes*, does exhibit expansigeny, *Nymphoides* roots usually do not; they exhibit schizogeny or schizo-lysigeny.

Diaphragms are found in *Thalassia* of the Hydrocharitaceae (Alismatales, monocots) where they were reported to be multiseriate (Tomlinson, 1969). In other Hydrocharitaceae, single strands of diaphragm cells can be found to traverse lacunae. It is anticipated that other plants with diaphragms of various kinds will be found; Seago (2002) had not found them in the Cabombaceae, but we confirmed their production. Another feature associated with species with expansigenous aerenchyma is that, because the lacunae arise in the root tip so near the RAM before lateral root initiation, lateral roots in plants such as *Acorus* traverse the aerenchyma without developing in any solid parenchymatous cortical tissue. In plant roots with other types of aerenchyma, lateral root primordia or bases are always surrounded by solid parenchymatous tissue (see, for example, Justin and Armstrong, 1987; Seago and Marsh, 1990; Soukup *et al.*, 2002).

DISCUSSION

The root cortex of wetland flowering plants is characterized by several patterns of development that normally result in aerenchyma tissue in the central regions of the cortex, as summarized in Fig. 5A–G, although there are wetland plant roots which do not have any aerenchyma tissue. We have determined that expansigeny produces honeycomb aerenchyma, the pattern of development and structure found in the basal angiosperms (Fig. 6). This is not a new or unique finding. We have confirmed the reports of Schenck (1890), Conard (1905), Němec (1907, 1930), Ellmore (1981) and Seago *et al.* (2000*a*, *b*) that patterns other than schizogeny and lysigeny exist in the root cortex.

RAMs in aerenchyma production

As described by Seago *et al.* (2000*b*) and confirmed here, the open RAMs of the Nymphaeales do not initiate a cortical organization involving radial files of cells across the ground meristem, even when the meristem may have a superficial closed and layered appearance, as in the Cabombaceae, as well as the Acoraceae of the basal monocots. The distal ground meristem has an irregular, non-radial appearance in transverse section (Fig. 5A), in contrast to many other plant roots (see Seago and Marsh, 1989; Seago *et al.*, 2000*b*). In many flowering plants, especially most monocots, only periclinal divisions in the proendodermis initiate the layers of the cortex, except where there is a hypodermis; anticlinal divisions (not oblique divisions) in cells along radii from the proendodermis produce any widening, mostly in the middle to outer portions of the central cortex to allow it to accommodate root expansion (see Clowes 1961; Seago and Marsh, 1989; Seago *et al.*, 2000*a*). Formation of a hypodermis generally involves a precise set of periclinal divisions in the outermost ground meristem (e.g. Seago and Marsh, 1989; Seago *et al.*, 2000*a*), and this region never has air spaces or aerenchyma.

The occurrence of oblique cell divisions in the cells of the mid-cortex, accompanied by cell expansions (Seago *et al.*, 2000*b*), leads to the enlargement of the intercellular spaces into lacunae that have a polygonal, hexagonal or honeycomb appearance. Even in plants with closed, tiered RAMs, in which the cortex arises from early periclinal divisions in the proendodermis, a precise set of oblique cell divisions initiated very near the RAM, followed by cell expansion instead of further schizogeny, can lead to honeycomb aerenchyma in quite widely scattered eudicot families (Fig. 6; see also Conard, 1905; Justin and Armstrong, 1987; Laan *et al.*, 1987). This seems somewhat unusual for plants with RAMs that are basically closed with one (or two) tier of cortical initials, but there must be a constitutive or genetic predisposition to such cell divisions in some plant species among eudicots, especially when the roots are subjected to hypoxia (Justin and Armstrong, 1987; Laan *et al.*, 1987).

The relationship between expansigeny and molecular, physiological and environmental factors influencing expansion needs to be explored; most research on the factors controlling the production of lacunae in aerenchyma has dealt with lysigenous aerenchyma (see, for example, Jackson and Armstrong, 1999; Evans, 2004). To understand how basal angiosperms arose, succeeded and remain the dominant and characteristic plants of some wetlands, species with expansigenous honeycomb aerenchyma should be examined to broaden our understanding of regulatory mechanisms controlling growth and survival of basal angiosperms in anoxic environments.

In addition to finding honeycomb aerenchyma in the Acorales, the fact that we found it in the Alismatales should not be surprising because they have such a wide variety of adaptations in their root cortices, and the Alismatales are considered the closest to the Acorales in the monocotyledonous lineage (Angiosperm Phylogeny Group, 1998, 2003; Chase, 2004; Soltis and Soltis, 2004); further, the Orchidaceae are basal in the Asparagales lineage (Angiosperm Phylogeny Group, 2003; Chase, 2004). We anticipate that future research will discover more expansigeny in the Alismatales and Asparagales, as well as in the Ranunculales (Fig. 6; see Justin and Armstrong, 1987; Seago *et al.*, 2000*b*) and Proteales of the basal Eudicots (Soltis and Soltis, 2004). We also need to know if honeycomb aerenchyma can arise by other developmental paths.
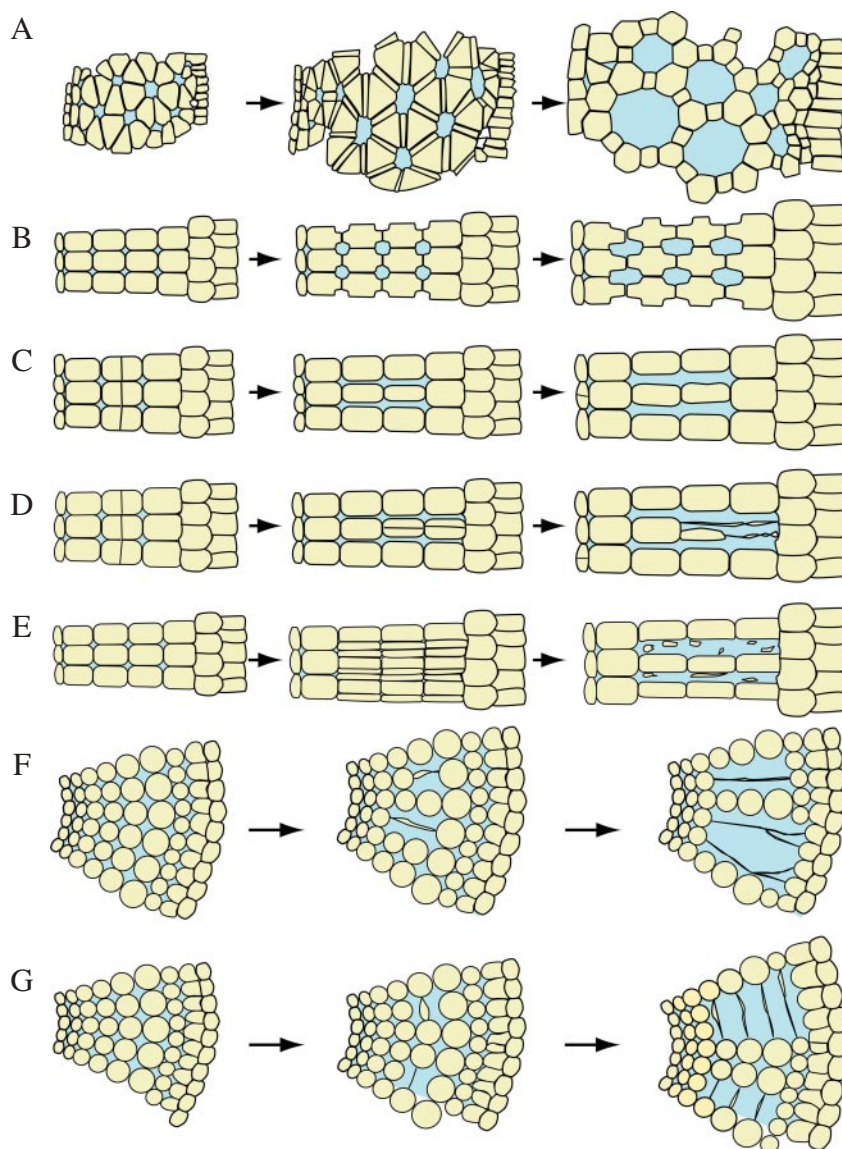
FIG. 5. Diagrams of aerenchyma types: representation from ground meristem stage to aerenchyma to mature stages; (A) honeycomb expansigeny; (B) radial expansigeny; (C) schizogeny; (D) schizo-lysigeny; (E) packet lysigeny; (F) radial lysigeny; (G) tangential lysigeny. The endodermis is always the layer of cells on the left, and outermost layer of the hypodermis (usually an exodermis; there are no spaces between hypodermal cells) is on the right in each set of drawings. Intercellular spaces and lacunae are coloured light blue.

Radial expansigeny (Fig. 5B) obviously has a constitutive basis and seems to be a variation or elaboration of the expansigeny producing honeycomb aerenchyma, and we expect to discover more families characterized by it. In the monocots, it seems that radial expansigeny would probably occur in plants phylogenetically more closely related to the Acorales and Alismatales than to the Poales, as is the case for the Commelinales (see Angiosperm Phylogeny Group, 2003).

In the Nymphaeales (Nymphaeaceae *vs*. Cabombaceae) and Asterales (Menyanthaceae), sclereids are present in roots growing in muddy substrates, but not in roots growing in water (Seago, 2002; Seago and Seago, 2004). Mechanical support or defence functions may be associated with these sclereids. However, it is the apparent developmental plasticity of sclereids not forming in roots growing in open water as opposed to forming in roots submerged in mud or other saturated substrate that is intriguing and needs to be tested experimentally.

The occurrence of uniseriate diaphragms across lacunae may be restricted to the Nymphaeales, and it raises a question about their development from the unique kind of RAM found in the Nymphaeales. The manner in which they arise as protuberances into enlarging lacunae may be related to the pattern of cell divisions which increase the numbers of cortical cells around the intercellular spaces and developing lacunae. The occurrence of various kinds of diaphragms in the Hydrocharitaceae (Alismatales) indicates that more species with diaphragms should be found.
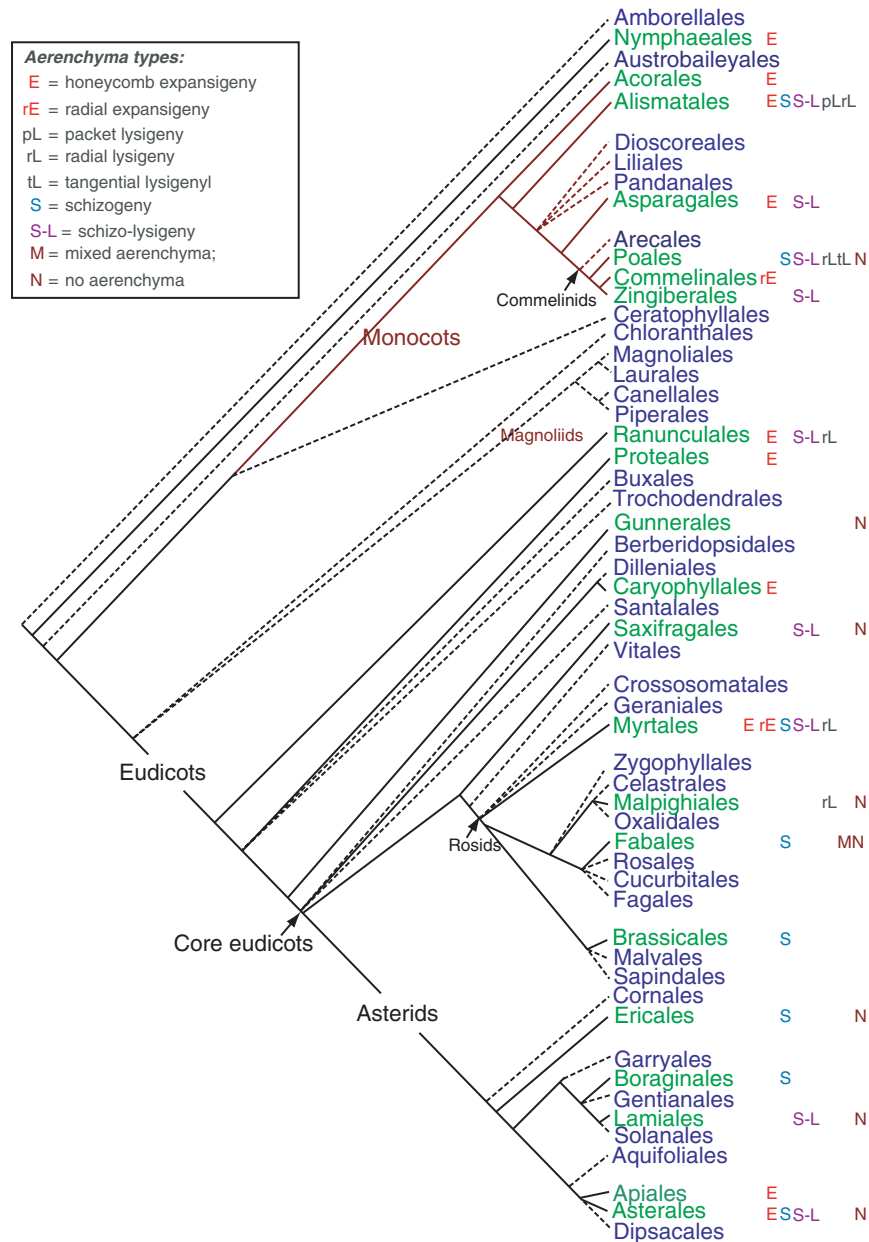
FIG. 6. The distribution of patterns of aerenchyma in angiosperms. The angiosperm phylogeny tree is modified from Angiosperm Phylogeny Group (2003) and with permission of Dr Peter F. Stevens. The solid lines lead to orders in green type that are covered in this study; the colour-coded aerenchyma types are listed next to these orders.

Schizogeny/schizo-lysigeny/lysigeny/mixed aerenchyma/ no aerenchyma

The plants characterized by schizogeny (Fig. 5C), mixed aerenchyma and schizo-lysigeny (Fig. 5D) cover a broad spectrum of monocots and eudicots (Fig. 6), and some roots normally without aerenchyma may occasionally have aerenchymatous spaces. We simply do not know in which groups of flowering plants schizogenous lacunae might have first arisen.

In legumes, the type of open RAM (transversal meristem, J. L. Seago, Jr, unpubl. res.; Clowes, 2000), the lack of an exodermis and the occurrence of nodules could be limiting

to developmental modifications in the ground meristem, and thus only some scattered schizogeny and mixed aerenchyma would be likely to occur in the cortex. Their roots are thus more open to aerenchymatous modification through secondary growth, i.e. the induction of secondary or peridermal aerenchyma (e.g. Justin and Armstrong, 1987; James and Sprent, 1999) under flooded conditions (e.g. James and Sprent, 1999; Shimamura *et al.*, 2003). There are strong indications that many perennial species in wetland habitats probably have radial expansigeny in the periderm (see Schenck, 1890; Stevens *et al.*, 2002). These situations need to be investigated further.

Lysigeny (Fig. 5E, F and G) may have originated in flowering plant roots which have closed RAMs with one tier of cortical–epidermal initials (monocots) or one tier of cortical initials (eudicots; see Clowes, 2000). This is particularly prevalent among the Alismatales and Poales in the monocots, and the Myrtales and Asterales of the eudicots. The radial orderliness of the ground meristem and cortex provides a framework for lysigeny with its attendant schizogeny.

The type of unusual lysigeny (Fig. 5E) found in the Araceae and Hydrocharitaceae of the Alismatales (Seago *et al.*, 1999*a*), involving redivided ground meristem cells along radial files of cells that lyse to create lacunae, may not be so unusual and may be expected in other monocots, especially in other Alismatales families; Tomlinson's (1969) description of lysigeny in *Thalassia* was not detailed enough to categorize.

Clearly, the very distinctive tangential lysigeny produced only by members of the Cyperaceae (Figs 5G and 6) is constitutive. This has been observed by numerous researchers engaged in various types of research on cyperaceous roots (e.g. Bristow, 1975; Smirnoff and Crawford, 1983; Končalová *et al.*, 1993).

Our interpretations of aerenchyma do not agree with those of Justin and Armstrong (1987) for all plants, especially for some species with honeycomb aerenchyma, but they do for most schizogeny and lysigeny. However, analyses of development enabled us to relate patterns of cell division and arrangements in RAMs to aerenchyma types; these are the factors that produced the cell packing arrangements noted by Justin and Armstrong (1987).

As noted previously, we observed an absence of aerenchyma from roots of carnivorous bog plants. However, it should be noted that Metcalfe and Chalk (1951) did not report any aerenchyma in their anatomical survey, including members of the Droseraceae and Sarraceniaceae. Perhaps low pH conditions affect cortical development, but more species need to be examined. The absence of aerenchyma in *Gunnera* and *Euphorbia* is not surprising because they lack cortex with radial cell files.

Systematic distribution of aerenchyma types

Figure 6 illustrates the distribution of various types of aerenchyma across the flowering plants. The early wetland flowering plants, the Nymphaeales of the basal angiosperms and the Acorales of the monocots, are characterized by expansigeny and honeycomb aerenchyma, but such aerenchyma is also found in scattered groups of eudicots and two groups of monocots, Alismatales (*Najas*, Hydrocharitaceae) and Asparagales (*Habenaria*, Orchidaceae). We fully anticipate that other types of aerenchyma may be found in the roots of flowering plants, because the two orders of eudicots (Myrtales and Asterales) and one order of monocots (Alismatales) have so many families and genera that are wetland plants (Arber, 1920, Sculthorpe, 1967; Bristow, 1975; Smirnoff and Crawford, 1983; Justin and Armstrong, 1987; Les and Schneider, 1995) that we may have barely begun to characterize aerenchyma types and distribution.

The special monocot situation

Les and Schneider (1995) have reiterated the argument that monocots had an aquatic origin, and the Acorales have consistently been shown to be the basal monocots (Angiosperm Phylogeny Group, 1998, 2003; Chase, 2004; Soltis and Soltis, 2004). We have now shown clearly that, with regard to basic aerenchyma development and root organization, the basal monocot, *Acorus calamus* (Acoraceae, Acorales), and certain Alismatales and Asparagales have expanisgenous honeycomb aerenchymas like the Nymphaeales (see also Němec, 1907). The diverse aquatic monocots appear to exhibit an adaptive radiation into a variety of aerenchyma types. Further, there is the seemingly unique situation in which various ranks of adventitious roots produced during the growing season have different patterns of aerenchyma (see Seago and Marsh, 1989; Seago *et al.*, 1999*b*); this may not be unusual at all for plants in which waves of adventitious roots are functionally replacement roots. Clearly, there is more to be learned about the roots of monocots.

Support in the fossil record

Some of the earliest fossil angiosperms of 90–125 million years ago appear to be wetland plants with similarities to the Nymphaeales (see Friis *et al.*, 1999, 2000, 2001; Crane *et al.*, 2004; Crepet *et al.*, 2004). Friis and her colleagues (Friis *et al.*, 1999, 2000, 2001; Mohr and Friis, 2000) have presented evidence that water lilies are found in the upper Jurassic/early Cretaceous in wetland environments. Further, the finding that the enigmatic *Archaefructus* is probably a water lily-like plant in its general morphology and lived in aquatic/wetland habitats (Sun *et al.*, 2002; see Crepet *et al.*, 2004) adds to the notion that early flowering plants were wetland plants or were at least well represented by aquatic or wetland species. If the ancient Nymphaeales and *Archefructus* had roots at all similar to the extant water lilies (Conard, 1905; Seago *et al.*, 2000*b*; Seago, 2002), then it becomes clear that, among the early flowering plants, there were aquatic plant groups which undoubtedly had aerenchymatous roots adapted to anoxic conditions. The expansigenous honeycomb aerenchyma in extant Nymphaeales is clearly the dominant feature of basal flowering plant roots, but, unfortunately, we simply do not know what the root cortex structure was like in those fossil organisms. We anticipate that it will prove to be honeycomb aerenchyma, because the expansigenous cell division and cell expansion pattern appears too basic to have evolved from a schizogenous or lysigenous precursor.

Ecological and evolutionary ramifications

It appears that aerenchyma formation had adaptive value for wetland flowering plants, including the earliest wetland flowering plants, many of which were obligate aquatic plants. Further, while they flower, wetland plants are often clonal and produce adventitious roots, especially the monocots which include both completely aquatic as well as amphibious species (Sculthorpe, 1967; Grace, 1993; Les and Schneider, 1995; Crow, 1999; Friis *et al.*, 1999, 2001;

Santamaria, 2002). Thus, the early adaptation of flowering plants to their wetland habitats was accompanied and made successful by the production of aerenchyma, allowing plants to succeed in such hypoxic or anoxic environments. Particularly in the monocots, seed germination in many of these wetland flowering plant communities is either rare or primarily replaced by clonal reproduction (see Grace, 1993), in which the roots systems are adventitious. We therefore suggest that the role of seed production is to colonize areas outside the established stands where less competition or more favourable conditions are conducive to seedling growth.

Assuming that there was an aerenchymatous system present in the shoot organs of early angiosperms, such as the extant Nymphaeales (Schneider and Williamson, 1993; Williamson and Schneider, 1993) and Acorales (Keating, 2003), an aerenchymatous system taking oxygen directly to the meristem cells may have been an evolutionary advantage. Further, eudicots that have expansigeny derived from closed RAMs might have certain advantages as amphibious plants in sudden floods because they are able to produce aerenchyma so close to their closed RAMs, thus maintaining root elongation and function (Laan *et al.*, 1987). However, in plants with schizogenous and lysigenous aerenchymas, even though intercellular spaces clearly arise within the initials region of root tips, their lacunae are farther removed from the meristem. Lysigeny, in particular, may be favoured in most of these plants because they have fewer living cells, greater porosity and lower energy demand (Justin and Armstrong, 1987, 1999; Evans, 2004) or because new adventitious roots, effectively replacement roots (Seago and Marsh, 1989; Seago *et al.*, 1999b), are formed. Such lysigenous lacunae might be associated with the cessation of root elongation and of active uptake of water and minerals in such roots (Longstreth and Borkhesenious, 2000).

Obviously, unique expansigenous features had evolutionary and ecological consequences for plants that had not evolved mechanisms for schizogeny and lysigeny. This could mean that there are in extant Nymphaeales, or were in extinct water lilies, environmental cues, hormonal responses, receptors or enzymes produced by these plants which are different from those we find in schizogenous and lysigenous systems, or precursor systems may be present.

CONCLUSIONS

We conclude that the development of intercellular spaces into lacunae to form root cortex honeycomb aerenchyma in aquatic angiosperms by expansigeny, not by schizogeny or lysigeny, was not an evolutionary accident, but coupled with contiguous and concomitant aerial shoot aerenchyma was an essential adaptive feature of early flowering plants, making possible aerobic life in anaerobic environments, and now aerenchyma development is the prevalent adaptation in most aquatic and wetland flowering plants. Continuous aerenchymatous systems from the aerial environment to the rhizosphere confer numerous other advantages, such as favourable substrate aeration and chemistry, useful microbial growth, diminished toxicity of heavy metals and, in some, pressurized ventilation, all of which need to be re-examined in light of our findings that the earliest flowering plants undoubtedly produced expansigenous honeycomb aerenchyma to survive the anaerobic conditions of their environments.

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