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# EVOLUTION OF ENDOSPERM DEVELOPMENTAL PATTERNS AMONG BASAL FLOWERING PLANTS

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A phylogenetically based comparative investigation of endosperm development was undertaken in a sample of 13 basal angiosperm taxa. The specific goals were to (1) provide a full developmental analysis of all aspects of endosperm in each species, (2) compare patterns among taxa to determine phylogenetic character distribution, (3) reconstruct the ancestral developmental pattern for angiosperms, and (4) explore scenarios of ontogenetic evolution that occurred during the early radiation of flowering plants. Five taxa, *Acorus calamus*, *Cabomba caroliniana*, *Ceratophyllum demersum*, *Drimys winteri*, and *Platanus racemosa*, are described in detail. Data from an additional eight taxa were analyzed and compared with these five. Endosperm ontogeny can be conceived of as a series of stages (characters) during which differential patterns of development occur among taxa (character states). We discovered that differential developmental fate of chalazal and micropylar domains is a common pattern among the endosperms of all basal angiosperm taxa and suggest that this may be a feature of endosperm development in all angiosperms. Differential development of chalazal and micropylar domains in endosperm in basal angiosperms also bears a marked similarity to what occurs in angiosperm embryos. This may have implications for understanding the evolutionary origin of endosperm. Basal angiosperms also exhibit variable endosperm developmental characters, indicating that significant ontogenetic transformation occurred during the early radiation of the clade, although magnoliid taxa exhibit a high degree of conservation in endosperm characters. Identification of the roles of the division of the primary endosperm nucleus and subsequent development of the chalazal and micropylar domains provides the first insight into how different endosperm developmental patterns are evolutionarily and developmentally related.

*Keywords:* angiosperm embryology, ontogeny, character evolution, endosperm, development, *Acorus*, *Cabomba*, *Ceratophyllum*, *Drimys*, *Platanus*.

## Introduction

The origin and early evolutionary history of flowering plants remains one of the seminal events in the history of life and one of the most enigmatic puzzles in evolutionary biology. The fossil record indicates that within a relatively short period of time, 10–12 million years, all major lineages of flowering plants (magnoliids, monocots, and eudicots) were established, and that within another 15 million years (during the Albian period) flowering plants became the most species-rich group of plants on Earth (Crane et al. 1995; Wing and Boucher 1998). This rapid diversification, along with the wide morphological gaps separating angiosperm reproductive structures from those of other seed plants, have combined to hinder our understanding of the origin and early evolution of angiosperms.

Fortunately, recent heightened interest in the early evolution of flowering plants has produced important new contributions to our knowledge of the reproductive characters of the earliest diverging extant angiosperms. Much has been learned about floral morphology (see, e.g., Endress 1987*a*, 1994, 1995; Friis and Endress 1990; Erbar and Leins 1994; Tucker and Bourland 1994; Williamson and Schneider 1994; Friis 1996; Tucker and Douglas 1996; Endress and Igersheim 1997, 2000*a*, 2000*b*; Igersheim and Endress 1997, 1998; von Balthazar and Endress 1999; Hayes et al. 2000), pollen structure (Sampson and Endress 1984; Foreman and Sampson 1987; Sampson 1987, 1993, 1995, 1996, 1997, 2000; Gabarayeva 1991; Osborn et al. 1991; Gabarayeva and Rowley 1994; Gabarayeva and El-Ghazaly 1997), and pollination biology (Thien 1980; Bernhardt and Thien 1987; Pellmyr et al. 1990; Schneider et al. 1990; Lloyd and Wells 1992; Williams et al. 1993; Ma et al. 1997; Allain et al. 1999; Bernardello et al. 1999; Dieringer et al. 1999; Luo and Li 1999; Thien et al. 2000). There have also been significant new findings and interpretations of early angiosperm fossils, which have altered our views of the time of origin, pace of diversification, and floral structure of the earliest angiosperms (Walker and Walker 1984; Doyle and Hotton 1991; Friis et al. 1991, 1994, 1995, 1997, 1999, 2000; Crepet et al. 1992; Crane et al. 1994, 1995; Crepet and Nixon 1994; Brener 1996; Crane and Herendeen 1996; Gandolfo et al. 1998; Sun et al. 1998; Lupia et al. 1999; Mohr and Friis 2000).

Despite significant recent progress in the assessment of many aspects of reproductive character distribution among basal lineages of flowering plants, some of the most defining angiosperm features have been largely overlooked. Embryological

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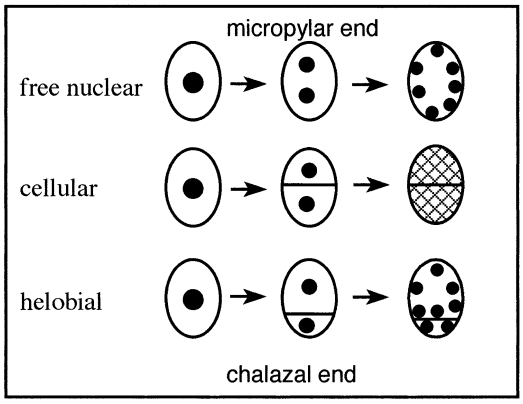


**Fig. 1** Diagram of the three traditionally recognized endosperm development types. The micropylar side is at the top; chalazal is at the bottom. Each developmental pattern begins with the primary endosperm nucleus in the first endosperm cell (former central cell) and follows the nuclear divisions and formation of cell walls.

characters are among the unique reproductive traits that separate angiosperms from all other seed plants. Features such as the reduced female gametophyte, a triploid second fertilization event, and the embryo-nourishing endosperm have long been recognized as synapomorphies of flowering plants. However, little is known about the development and evolution of these characters in basal lineages. Data are simply lacking for many taxa (Bhandari 1971). In addition, more recent investigations of endosperm development in a few early-diverging angiosperms (Hayashi 1963; Bhandari and Venkataraman 1968; Tobe et al. 1993; Floyd et al. 1999; S. Floyd, unpublished data) have revealed that previous reports of developmental patterns in these taxa were erroneous. Given the claimed importance of the origin of many aspects of embryology, particularly endosperm, to the "success" of flowering plants (Stebbins 1976; Tiffney 1981), this lack of basic information constitutes a serious gap in our overall knowledge of the basic biology of the most dominant and diverse group of plants on Earth.

In addition to a fundamental lack of embryological data for basal flowering plants, the angiosperm embryological literature has failed to provide clear hypotheses of the ontogenetic evolution of endosperm among angiosperms (Gifford and Foster 1989). There are two major reasons for the current lack of understanding of endosperm evolution: (1) a reliance on typology for descriptions of complex developmental patterns and (2) a largely nonphylogenetic approach to the study and comparison of the diversity of endosperm patterns to be found among angiosperms.

For most of the twentieth century, descriptions of complex ontogenetic patterns associated with female gametophyte, embryo, and endosperm development have been reduced to simple typological categories. This is perhaps most striking in the case of endosperm, for which three types of development (fig. 1) are employed in the categorization of endosperm in over 250,000 species of angiosperms. Free nuclear development encompasses all patterns in which the triploid primary endosperm nucleus (the product of the second fertilization event) divides without formation of a permanent cell wall. Typically, additional mitotic divisions without cytokinesis follow. The result is a coenocytic endosperm, which may later become cellularized. *Ab initio* cellular endosperm results when cell walls are formed following the first and all subsequent divisions of the primary endosperm nucleus and its derivatives. In helobial endosperm, a pattern common among monocot taxa (Swamy and Parameswaran 1962), division of the primary endosperm nucleus is followed by formation of a cell wall that unequally partitions the endosperm into two cells. Free nuclear development then occurs in at least one of these cellular compartments.

A major shortcoming of the typological scheme for endosperm is that each type focuses on only one or two aspects of early endosperm ontogeny (fig. 1). For example, "free nuclear" indicates only that there is an initial free nuclear phase but does not provide information about the duration of free nuclear proliferation (one to many rounds of free nuclear divisions), whether or not the coenocytic endosperm ultimately cellularizes, and, if so, how cellularization occurs. The extent and nature of cellular differentiation is entirely overlooked (Floyd et al. 1999). Variation in endosperm ontogeny among taxa classified as "helobial" has been well documented and includes differences in the nature and extent of development in the two initially established cells, as well as whether or not cellularization occurs in coenocytic cells (Swamy and Parameswaran 1962; Grayum 1991). Yet the designation "helobial" only indicates that an initial cellular partitioning is followed by some free nuclear development. Similarly, consid-

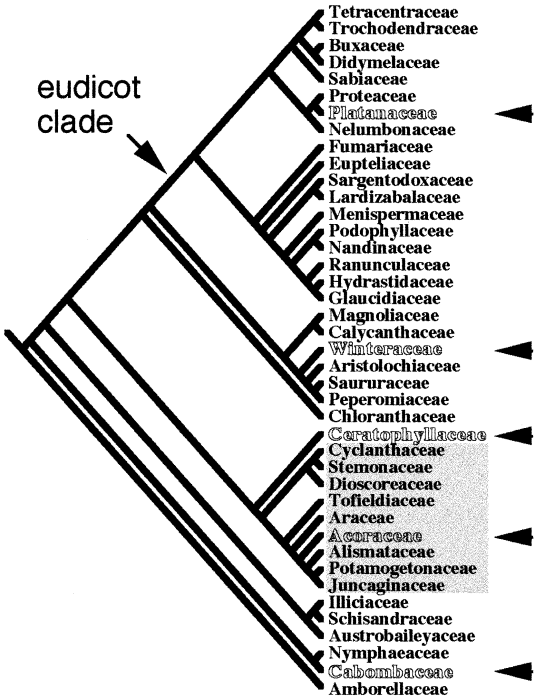


**Fig. 2** Phylogenetic placement of the five taxa that are discussed in detail in this article, *Acorus*, *Cabomba*, *Ceratophyllum*, *Drimys*, and *Platanus*. The shaded box indicates the monocot clade. Tree based on Qiu et al. (1999).

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**Table 1**

**Source and Voucher Information for the 13 Basal Angiosperm Species in This Study**

| Taxa | Source | Herbarium voucher or garden accession number |
|---|---|---|
| *Acorus calamus* ..... | Ann Arbor, Mich. | COLO Floyd 97-53 |
| *Amborella trichopoda* ..... | National Tropical Botanical Garden; Leonard Thien, Tulane University | Lorence 8346 (PTBG) #1000 in liquid specimen |
| *Austrobaileya scandens* ..... | University of Zurich Botanic Garden | P. K. Endress 98-47 |
| *Cabomba caroliniana* ..... | San Marcos, Tex. | COLO Floyd 97-57 |
| *Calycanthus floridanus* ..... | Georgia State Botanical Garden | COLO Floyd 97-40 |
| *Ceratophyllum demersum* ..... | San Marcos, Tex. | COLO Floyd 97-58 |
| *Drimys winteri* ..... | Cultivated, University of Colorado, Boulder, 30th Street Greenhouse | 92.001 |
| *Illicium mexicanum* ..... | Cultivated, University of California Botanic Garden, Berkeley | 91.0030 |
| *Liriodendron tulipifera* ..... | University of Georgia campus | COLO Floyd and Williams 97-30 |
| *Platanus racemosa* ..... | Redlands, Calif. | Floyd and Swan 97-47 |
| *Sarcandra chloranthoides* ..... | Cultivated, University of Zurich Botanical Garden | (Z-ZT): Maria von Balthazar 1 |
| *Sarcandra glabra* ..... | Cultivated, Missouri Botanical Garden | MBG#952650 |
| *Saururus cernuus* ..... | Ft. Stewart, Ga.; Athens, Ga. | Floyd 97-34 (liquid specimen) Floyd 97-62, 97-64, 97-69, 97-72 (liquid specimen) |
| *Schisandra sphenanthera* ..... | Cultivated, University of Zurich Botanical Garden | No collection number |

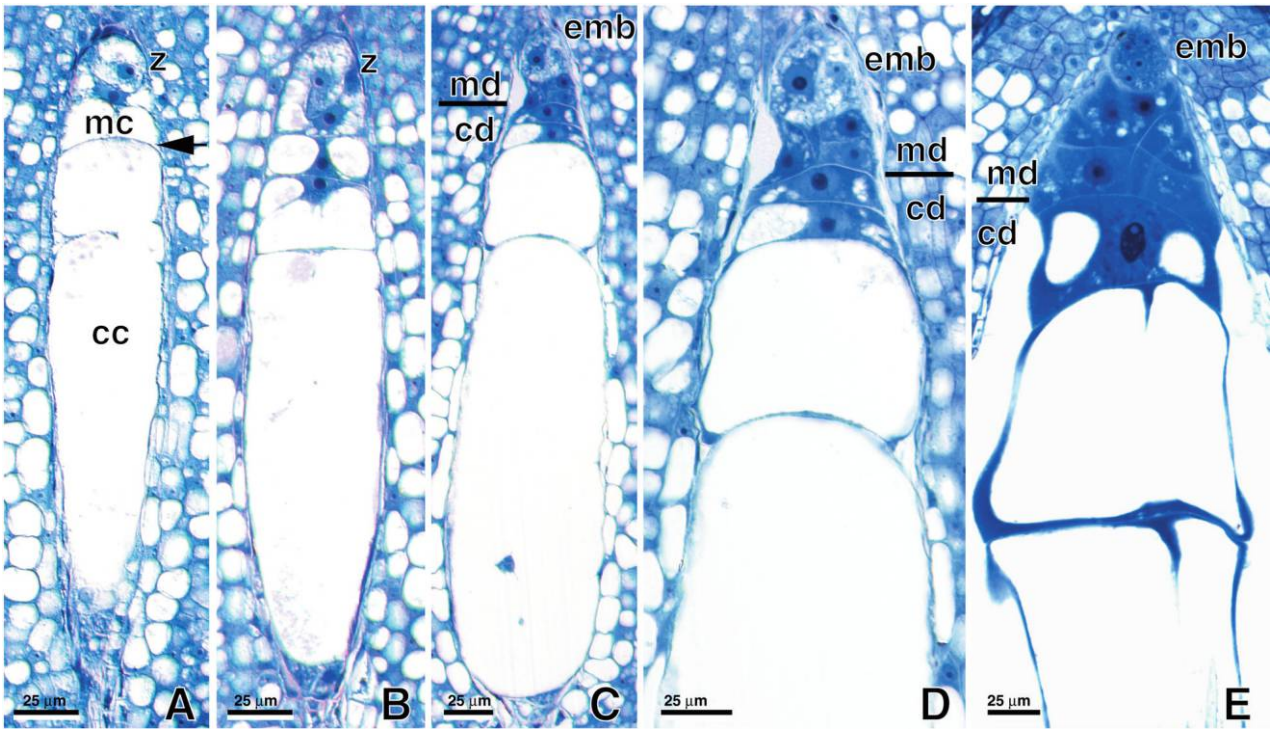


**Fig. 3** Light micrographs showing endosperm development in *Ceratophyllum demersum*. All sections are longitudinal and oriented with the micropylar end at the top of the page. The first transverse wall is indicated by the arrow. *A*, Two-celled endosperm stage. The first endosperm cell has been partitioned unequally into a large chalazal cell (*cc*) and a smaller micropylar cell (*mc*); zygote (*z*) is present at micropylar end of endosperm. *B*, Four-celled endosperm. Each of the first two cells has been partitioned by a transverse wall, resulting in a four-celled uniseriate endosperm; zygote (*z*) remains undivided. *C*, Endosperm differentiated into a chalazal domain (*cd*), four large vacuolate cells in a uniseriate arrangement, and a micropylar domain (*md*), a multidimensional mass of cells adjacent to the two-celled embryo (*emb*). *D*, Same endosperm shown in *C* at higher magnification. *E*, Most advanced endosperm stage, with the enlarged uniseriate chalazal domain (*cd*) and the globular multicellular (21 cells) micropylar domain (*md*) adjacent to the globular embryo (*emb*).

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erable developmental variation is reported by independent investigators who have studied *ab initio* cellular endosperm (Schnarf 1929; Maheshwari 1950; Dahlgren 1991), although the label "*ab initio* cellular" implies nothing more than cytokinesis coupled with karyokinesis during development. This typological framework has rendered the embryological literature of limited use for the reconstruction of the ontogenetic evolution of endosperm.

Clearly, various embryologists have reported observations of development that go beyond typological designation (Davis 1965; Bhandari 1971; Grayum 1991; Johri et al. 1992). There has, unfortunately, been no consistent framework for the description of endosperm developmental features among major taxa. The result is that it is nearly impossible to make meaningful developmental comparisons that transcend typology across different taxonomic groups. We argue that this too is the consequence of "typological thinking" that tends to limit the objectives and results of embryological research.

Because endosperm ontogeny has been reduced to a single character with three states (free nuclear, *ab initio* cellular, and helobial), there is no developmental basis for understanding evolutionary relationships among the three patterns (Gifford and Foster 1989). We will demonstrate that endosperm development is a complex process involving discrete and separable phases. Furthermore, a full developmental analysis of endosperm can provide many characters that may be compared among taxa. We will also show that with the identification of the "natural" components of endosperm development, it is possible to determine the independent characters that have evolved to produce different developmental patterns.

A critical key to reconstructing character evolution is the incorporation of a phylogenetically based approach. Understanding the origin and early evolution of endosperm depends upon correct determination of the plesiomorphic condition among angiosperms. Assessment of the evolutionary polarity of endosperm types has been the topic of discussion at various times in the twentieth century (Schnarf 1929; Maheshwari 1950; Sporne 1954; Swamy and Ganapathy 1957; Wunderlich 1959; Donoghue and Doyle 1989; Dahlgren 1991; Kapil and Bhatnagar 1991; Friedman 1994). The most widespread view in the first half of the twentieth century was that free nuclear endosperm represents the plesiomorphic condition for angiosperms. This conclusion was based on the common occurrence of cellular endosperm among derived taxa (e.g., members of the Asteridae) (Wunderlich 1959 and references therein). Sporne also concluded that free nuclear development was primitive based on its statistical correlation with other putatively primitive characters (Sporne 1954). Free nuclear endosperm has also been considered plesiomorphic because it is the most common type among angiosperms (Johri et al. 1992).

In contrast, all but one (Schnarf 1929) of the surveys of endosperm character polarity that examined character-state distribution in putatively "primitive" angiosperms (e.g., members of the Magnoliidae) concluded that *ab initio* cellular endosperm is plesiomorphic (Wunderlich 1959; Bhandari 1971; Dahlgren 1991; Kapil and Bhatnagar 1991; Takhtajan 1991). Similarly, recent phylogenetically based analyses that utilized explicit cladistic methods indicated that *ab initio* cellular endosperm is plesiomorphic among angiosperms and that other developmental patterns (free nuclear and helobial) are derived (Donoghue and Doyle 1989; Friedman 1994; Floyd et al. 1999). An explicitly phylogenetic approach to both the selection of taxa and analysis of character distribution is absolutely necessary for the assessment of character polarity and reconstruction of evolutionary transitions in endosperm development. Of course, the strength of any conclusions based on such an approach depends on the reliability of the phylogenetic hypothesis.

Fortunately, in the last decade considerable effort has been expended on the cladistic analysis of phylogenetic relationships of flowering plants (Donoghue and Doyle 1989; Hamby and Zimmer 1992; Chase et al. 1993; Qiu et al. 1993, 1999, 2000; Doyle et al. 1994; Nixon et al. 1994; Nandi et al. 1998; Soltis et al. 1998, 1999, 2000; Hoot et al. 1999; Mathews and Donoghue 1999; Parkinson et al. 1999; Doyle and Endress 2000; Graham et al. 2000), and this has resulted in tremendous improvement in our understanding of the relationships of basal angiosperms. Although topologies vary in significant ways with each analysis, within the last year, five independent laboratories have coalesced on the same result for the three earliest-diverging lineages of extant flowering plants (fig. 2) (Mathews and Donoghue 1999; Parkinson et al. 1999; Qiu et al. 1999; Soltis et al. 1999; Graham et al. 2000). With an emerging consensus about basal angiosperm relationships and the rooting of the tree, it is now possible for the first time to undertake a phylogenetically based approach to the analysis of endosperm that will conclusively resolve character polarity and identify character-state transitions during the earliest radiation of extant flowering plants.

Unfortunately, basic structural data for the endosperms of many basal angiosperm taxa are either lacking or are limited to more cursory typological categorization. Clearly, what is needed is a phylogenetically based comparative investigation of endosperm development in critical basal taxa without *a priori* assumptions or typological limitations. Only with this approach can we assess the fundamental plesiomorphic pattern of endosperm development in angiosperms and begin to infer the key ontogenetic transitions that occurred during the early radiation of flowering plants.

We initiated a comparative study of endosperm development of 13 angiosperm taxa. Species were selected to represent a phylogenetically diverse sample of basal angiosperms, including a basal eudicot (*Platanus*), *Acorus*, which may be the sister group to all other monocots (Chase et al. 1993; Duvall et al. 1993*a*, 1993*b*), several magnoliid taxa representing lineages now resolved as the ANITA grade and eumagnoliid clade (*sensu* Qiu et al. 1999, 2000), as well as Ceratophyllaceae and Chloranthaceae whose phylogenetic affinities are variously resolved in different analyses (see Qiu et al. 1999 and Soltis et al. 1999) (table 1; fig. 2). The specific goals were to (1) provide a full developmental analysis of all aspects of endosperm in each species, (2) compare patterns among taxa to determine phylogenetic character distribution, (3) reconstruct the ancestral developmental pattern for angiosperms, and (4) explore scenarios of ontogenetic evolution that occurred during the early radiation of flowering plants.

In this article we focus on endosperm development in five (of the 13) taxa, *Acorus calamus* (a basal monocot), *Cabomba caroliniana* (a member of the Nymphaeales, which are sister to all angiosperms except *Amborella*), *Ceratophyllum demer-*

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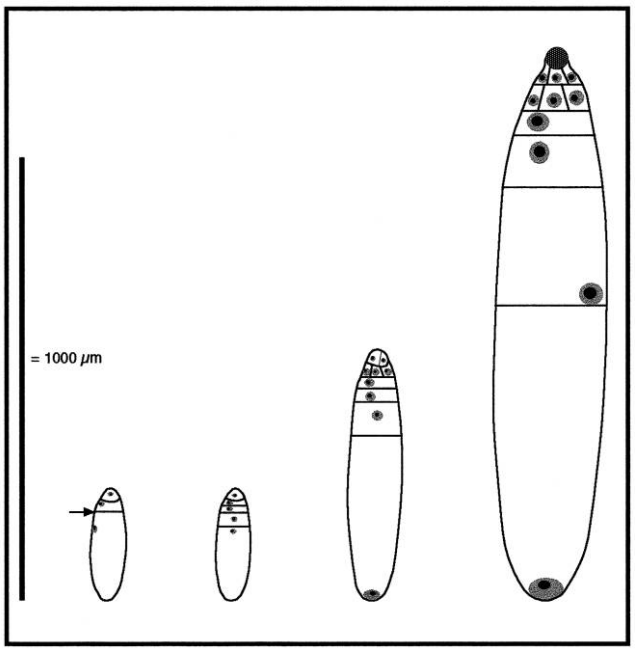


**Fig. 4** Diagrammatic summary of endosperm development in *Ceratophyllum demersum* corresponding to stages in fig. 2 and drawn to relative scale. Arrow indicates the location of the first transverse wall. Nuclei are shown for reference but are not accurately drawn to scale.

*sum* (a magnoliid of uncertain phylogenetic placement), *Drimys winteri* (Winteraceae), and *Platanus racemosa* (a lower eudicot) (hereafter referred to by their generic names only) (fig. 2). These five taxa were selected as exemplars not only because they represent a phylogenetically diverse sample but because they capture much of the breadth of basic endosperm developmental patterns observed in our analysis. *Acorus*, *Ceratophyllum*, and *Drimys* all have *ab initio* cellular types of endosperm; *Cabomba* has a helobial endosperm developmental pattern, and *Platanus* endosperm exhibits a free nuclear pattern. We describe the full developmental sequence of each taxon, compare patterns among taxa, and finally, explore patterns of character distribution in order to elucidate plesiomorphic endosperm features and ontogenetic transformations that occurred during the early radiation of the angiosperms.

## Material and Methods

### *Collections*

Reproductive material representing bud through fruit stages was collected from cultivated plants or individuals in wild populations for 10 species of basal angiosperms (table 1). Most specimens were placed in plastic bags, kept cool, and shipped for overnight delivery to the laboratory in Boulder, Colorado. *Drimys* was collected locally (from the University of Colorado greenhouse) and brought directly to the lab. Mature seeds for three additional species, *Amborella trichopoda*, *Austrobaileya scandens*, and *Schisandra sphenanthera*, which have been resolved among two of the three basal-most angiosperm lineages in recent phylogenetic analyses (Mathews and Donoghue 1999; Parkinson et al. 1999; Qiu et al. 1999; Soltis et al. 1999; Graham et al. 2000), were also obtained. In addition, a small number of buds, flowers, and developing fruits of *Amborella* (collected in New Caledonia) were provided by Leonard Thien, Tulane University.

### *Histology*

Materials provided by others (*Amborella*, *Austrobaileya*, *Schisandra*) were fixed in either formalyn acetic acid (FAA) or 70% ethanol and were stored in 70% ethanol. Flowers or carpels of all other taxa were placed into vials containing either 50 mM PIPES buffer (also 5 mM EGTA and 1 mM $MgSO_4$) at pH 6.8 or 100 mM PIPES buffer (also 10 mM EGTA and 2 mM $MgSO_4$) at pH 6.8. Acrolein was added to the vials with PIPES buffer to a concentration of 4% or 6%. Specimens were left in fixative a minimum of 48 h and then rinsed and stored in PIPES buffer at 4°C until needed.

Fixed specimens were dehydrated through an ethanol series to 95% ethanol, infiltrated with monomer A of the JB-4 embedding kit (Polysciences, Warrington, Pa.), and embedded in an oxygen-free environment. Blocks were serially sectioned at 5 μm on either a MICROM (Walldorf, Germany) or a Leica (Nussloch, Germany) rotary microtome using glass knives. Slides were stained in 0.1% toluidine blue, examined, and digitally imaged on a Zeiss (Carl Zeiss, Jena, Germany) Axiophot microscope equipped with a Zeiss Axiocam digital camera, using both bright field and differential interference contrast (DIC) optics.

The presence of proteins, lipids, and starch in endosperm and other tissues was examined with histological stains and the use of cross polarization microscopy. To visualize proteins, a 1% solution of naphthol blue black in 7% acetic acid was applied to unmounted slides prepared as above, which were then rinsed with acetic acid, dried, and mounted with cover glasses. The presence of lipids was determined by soaking hand sections of fresh seed material in 50% ethanol for 1 min, followed by the application of a saturated solution of Sudan IV in 70% ethanol for 10 min. The sections were then rinsed with 50% ethanol, mounted in glycerin, and observed. To test for the presence of starch, iodine-potassium iodide (IKI) was applied to hand sections of fresh material and slides (prepared as above) were observed under cross-polarized light.

Character distribution and estimation of ancestral and derived states were inferred using the program MacClade (Maddison and Maddison 1992). Characters were traced using the most parsimonious resolving option. The recent angiosperm phylogenies of Qiu et al. (1999) and Soltis et al. (1999) were used for character mapping for two reasons. Both analyses include a broad enough sample of magnoliid taxa to trace character transitions in the basal part of the angiosperm tree. Furthermore, they represent two of the published phylogenies that concur on the rooting of the angiosperm tree (Mathews and Donoghue 1999; Parkinson et al. 1999; Qiu et al. 1999; Soltis et al. 1999). The two phylogenies differ in several respects, including rooting of monocots and placement of Acoraceae, placement of Chloranthaceae, and position of Ceratophyllaceae. However, these topological differences have no effect on the inference of endosperm character polarity and character-state transitions discussed herein. For simplicity, we

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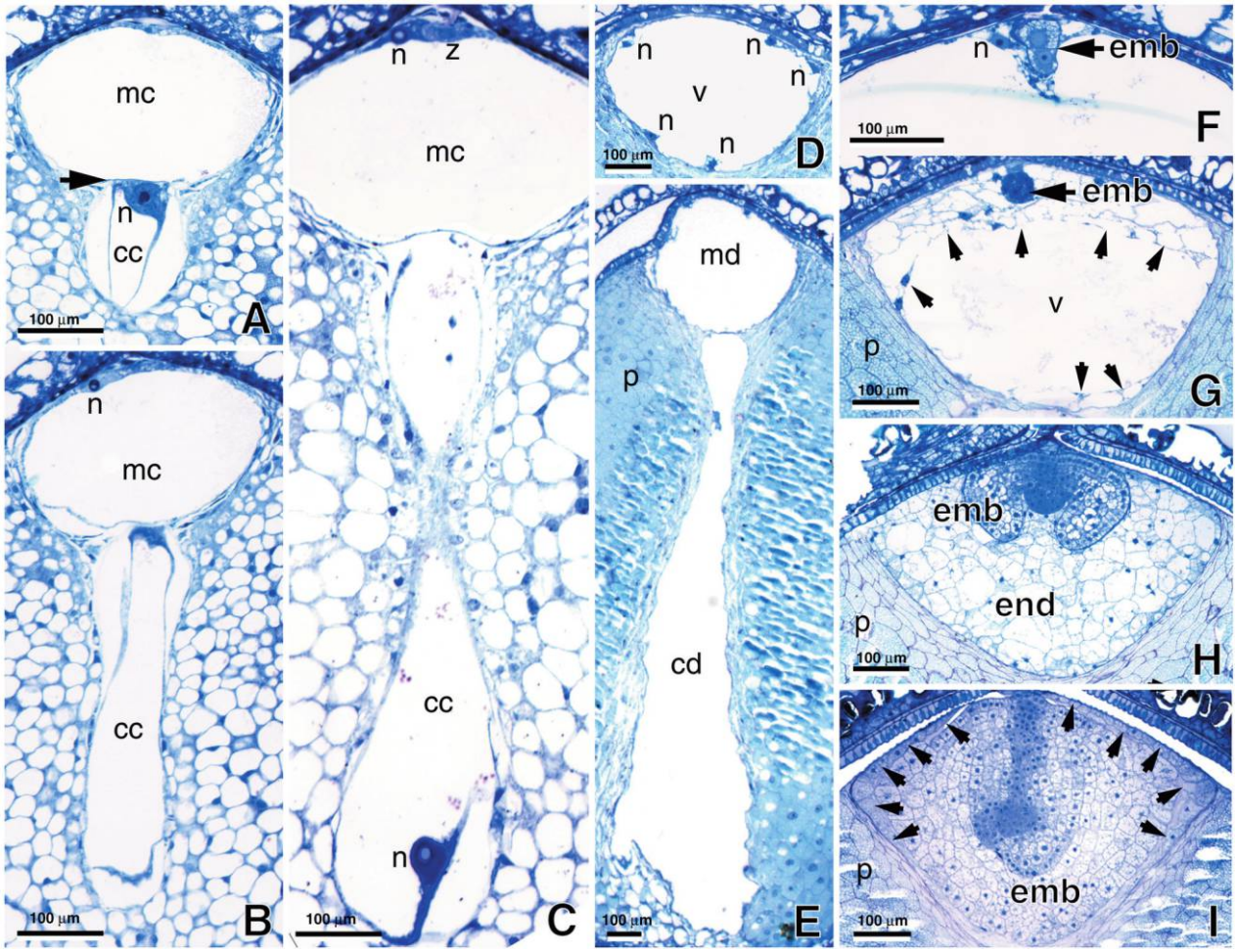


**Fig. 5** Light micrographs showing endosperm development in *Cabomba caroliniana*. All sections are longitudinal and oriented with the micropylar end at the top of the page. *A*, Two-celled endosperm stage. The first endosperm cell has been partitioned unequally into small chalazal (*cc*) and large micropylar (*mc*) cells. The first transverse wall is indicated by the large arrow, and the nucleus (*n*) of the chalazal cell is adjacent to the wall. *B*, Different section through the same endosperm in *A* showing the nucleus of the micropylar cell (*mc*). *C*, Two-celled endosperm at a slightly later stage than in *A* and *B*. The nucleus (*n*) of the chalazal cell (*cc*) has migrated to the chalazal end. The nucleus (*n*) of the micropylar cell (*mc*) is adjacent to the zygote (*z*). *D*, Free nuclear development has occurred in the micropylar cell, resulting in several free nuclei (*n*) around a large central vacuole (*v*). *E*, Entire endosperm at the free nuclear micropylar stage, with differentiated micropylar domain (*md*) and chalazal domain (*cd*). The chalazal cell has invaded the perisperm (*p*) as a unicellular uninucleate haustorial tube. *F*, Endosperm with free-nuclear micropylar domain showing one endosperm nucleus (*n*) adjacent to the two-celled embryo (*emb*). *G*, Centripetal cellularization occurring in the micropylar zone around the central vacuole (*v*). Walls (small arrows) have formed more rapidly at the micropylar end and have formed around the developing (globular) embryo (*emb*). *H*, Cellularization of the micropylar domain is complete. Cellularized micropylar endosperm (*end*), consisting of large vacuolate cells, surrounds the developing embryo (*emb*). *I*, Mature seed with embryo (*emb*) nearly filling the micropylar domain, surrounded by a thin layer of cellularized endosperm (small arrows).

present the characters mapped onto the topology of Qiu et al. (1999).

Although for brevity we include detailed developmental descriptions for five of 13 study taxa, our original data are included in tabular form and used in all comparative evolutionary analyses. In addition, data from the literature are included in all parsimonious character optimizations.

## Results

### *Ceratophyllum*

In *Ceratophyllum*, endosperm development begins with mitosis of the primary endosperm nucleus at the micropylar end of the first endosperm cell (the former central cell of the female gametophyte), near the zygote. After the nuclei move apart, a transverse wall is formed that partitions the first endosperm cell into a small micropylar cell and large chalazal cell (fig. 3*A*). The next stage of development frequently observed was a four-celled uniseriate endosperm, which is most likely the result of division of both cells in the two-celled endosperm but may result from two successive divisions of the micropylar cell and one of its derivatives (fig. 3*B*).

The "apical" endosperm cell at the micropylar pole of the four-celled endosperm divides transversely to produce a uniseriate stage with five endosperm cells. The micropylar-most

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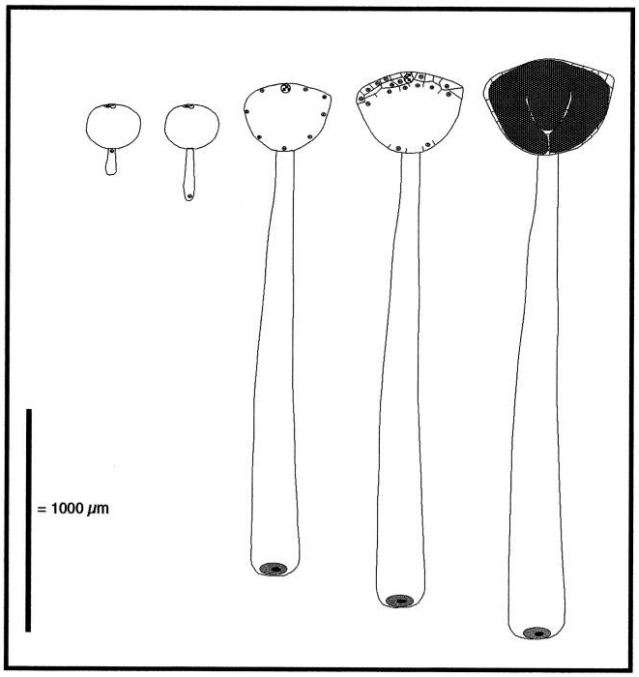


**Fig. 6** Diagrammatic summary of endosperm development in *Cabomba caroliniana* corresponding to most of the stages shown in fig. 5 and drawn to relative scale. Nuclei are shown for reference but are not accurately drawn to scale.

cell and its derivatives undergo a limited number of divisions in many planes to yield a variable number of cells (ca. 20) that become densely cytoplasmic (fig. 3*C*, 3*D*). The remaining four cells of the uniseriate stage enlarge without nuclear or cellular division (fig. 3*C*–3*E*). Small protein bodies are frequently observed in the cytoplasm of all endosperm cells.

The fully differentiated endosperm consists of four large highly vacuolate cells at the chalazal end and a small globular micropylar mass of about 20 cells (fig. 3*E*). We refer to these regions of differential development as the "chalazal domain" and the "micropylar domain." The mature endosperm occupies much of the developing seed volume, despite its limited cellular development. The embryo does not begin to develop until proliferation of the micropylar mass of endosperm cells has been initiated. The embryo then grows and displaces the endosperm, which becomes much reduced in volume. Endosperm cytoplasm and nuclei can still be observed near the apex of the embryo in mature seeds. The mature embryo, which occupies most of the seed volume, develops two large cotyledons and several nodes with whorls of leaf primordia. Starch is stored in the cotyledons and larger leaves. *Ceratophyllum* endosperm development is diagrammatically summarized in figure 4. Our findings are consistent with previous reports of endosperm development in *Ceratophyllum* (de Klercker 1885; Sastri 1955; Shamrov and Batygina 1984).

### *Cabomba*

Endosperm development in *Cabomba* begins with the primary endosperm nucleus located near the zygote. After division of the primary endosperm nucleus, a wall is formed that partitions the first endosperm cell into a small chalazal cell and a larger micropylar cell (fig. 5*A*–5*C*). The endosperm nucleus in the micropylar cell migrates to the micropylar end (fig. 5*B*, 5*C*) and initiates a few rounds of free nuclear divisions. These nuclei become distributed in a parietal layer of cytoplasm that surrounds the central vacuole of the micropylar cell (fig. 5*D*–5*F*). Small protein bodies are always present in the cytoplasm of the free nuclear micropylar cell. The chalazal cell enlarges and grows into the massive nucellus as a haustorial tube that sometimes branches (fig. 5*B*, 5*C*, 5*E*). The single nucleus migrates to the chalazal tip of the tube (fig. 5*C*) and enlarges but never divides.

Centripetal cellularization occurs around the periphery of the micropylar cell (fig. 5*G*), beginning first at the micropylar end. Ultimately the micropylar cell becomes completely compartmentalized into uninucleate cells (fig. 5*H*). At its peak of development, the endosperm occupies very little of the seed volume and is highly differentiated into a vacuolate chalazal domain and a globular multicellular micropylar domain. Embryo development is initiated early in the free-nuclear stage of the micropylar region (fig. 5*F*). After the micropylar chamber cellularizes, the embryo forms cotyledons and ultimately fills the micropylar chamber, replacing most of the cellularized endosperm (fig. 5*H*, 5*I*). At seed maturity, there is a small amount of endosperm surrounded by a massive perisperm (fig. 5*I*). Lipids (but not starch or proteins) were detected in the thin layer of endosperm that surrounds the embryo. Abundant starch grains are present in the perisperm. *Cabomba* endosperm development is diagrammatically summarized in figure 6.

### *Acorus*

Just after fertilization in *Acorus*, the primary endosperm nucleus is located midway between the micropylar and chalazal poles of the first endosperm cell. The primary endosperm nucleus migrates to the chalazal end where it divides. One daughter nucleus migrates a short distance toward the micropylar end, followed by the formation of a transverse wall that divides the endosperm into a smaller chalazal and larger micropylar cell (fig. 7*A*). The nucleus of the micropylar cell divides and another transverse wall is formed. Transverse cell divisions occur repeatedly, forming up to 14 large vacuolate cells (fig. 7*B*). In the chalazal cell, a vertical wall usually forms after the nucleus divides, although this varies. Cell divisions continue in various planes (fig. 7*B*). These early stages of differential development result in an endosperm that consists of a chalazal domain that is a multiseriate mass of small cells and a micropylar domain composed of a uniseriate arrangement of larger cells (fig. 7*B*). Following this differentiated endosperm stage, cell divisions in many planes occur also in the micropylar region (fig. 7*C*), resulting in a completely multiseriate endosperm.

The next stage of endosperm development in *Acorus* involves cell proliferation. Repeated cell divisions (in many planes) in both chambers produce a multicellular endosperm, composed of small uniformly sized cells, that occupies a significant portion of the seed volume (fig. 7*D*). During development, cells at the extreme chalazal end of the chalazal zone

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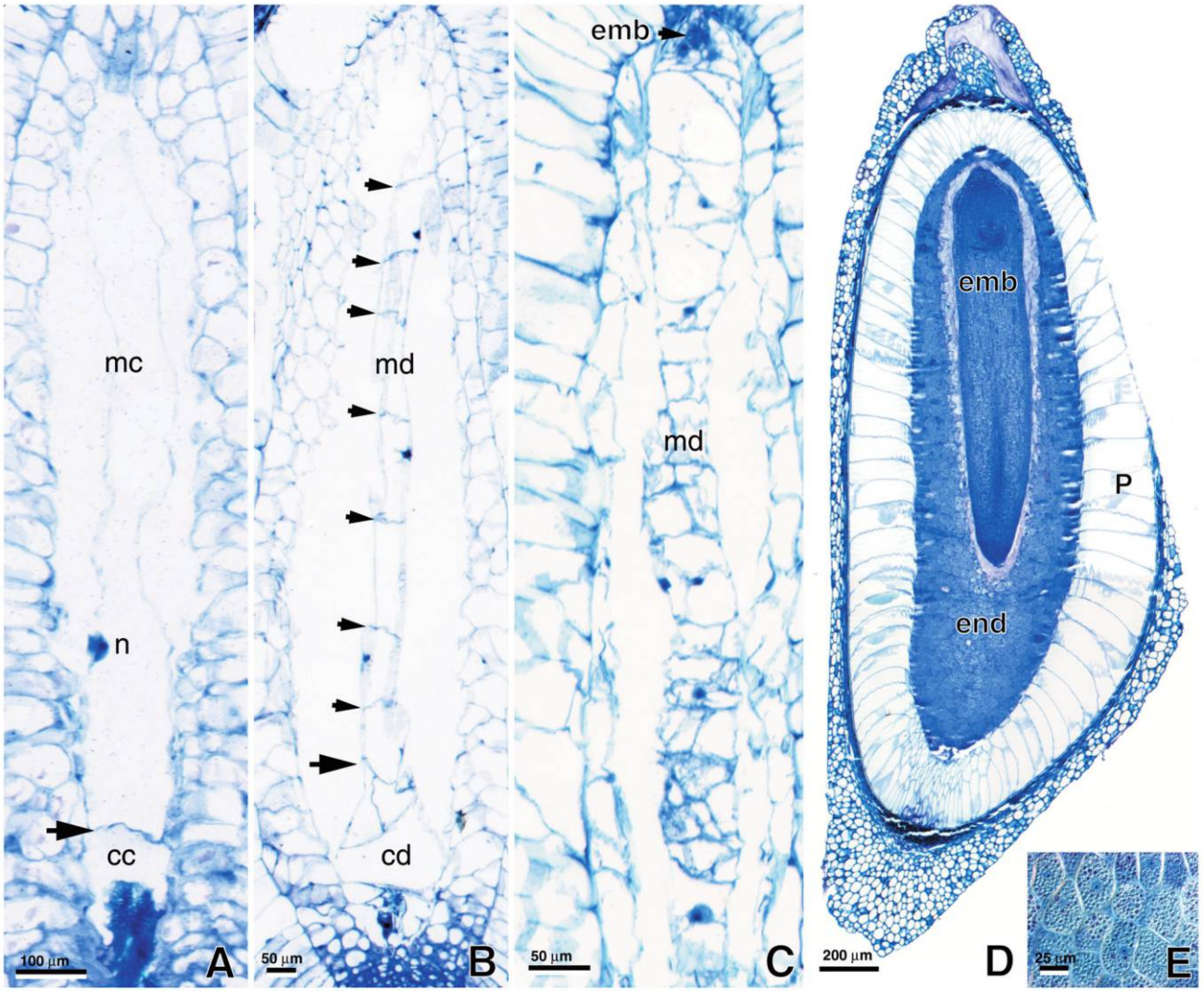


**Fig. 7** Light micrographs showing endosperm development in *Acorus calamus*. All sections are longitudinal and oriented with the micropylar end at the top of the page. *A*, Two-celled endosperm. First endosperm cell has been unequally partitioned by a transverse wall (arrow) into a large micropylar cell (*mc*) and a small chalazal cell (*cc*). *B*, Differential early development in the endosperm. Several divisions followed by the formation of transverse walls have resulted in a uniseriate micropylar domain (*md*). Cell divisions in various planes have led to a mass of smaller cells comprising the chalazal domain (*cd*). Large arrow indicates the initial transverse wall. *C*, Cell divisions in many planes have occurred in the micropylar domain (*md*). The embryo (*emb*) is in an early stage of development. *D*, Mature seed with well-developed embryo (*emb*), mature endosperm (*end*), and surrounding nucellar-derived perisperm (*p*). *E*, Mature endosperm cells filled with protein bodies and lipids.

are densely cytoplasmic, whereas the remaining endosperm cells are vacuolate and larger. All endosperm cells ultimately become densely cytoplasmic and fill with protein bodies and lipids (fig. 7*E*). At maturity, cells in the chalazal region are similar to those derived from the micropylar cell but tend to have thicker walls. Most of the endosperm is ultimately derived from the micropylar cell of the two-celled endosperm.

Embryo development begins during the early stages of differential endosperm development but does not acquire a globular morphology until after the micropylar zone has commenced cell division in many planes (fig. 7*C*). The embryo eventually develops a root apex, a shoot apex, and one large cotyledon. At seed maturity there is a moderate amount of endosperm surrounded by a perisperm (fig. 7*D*). *Acorus* endosperm development is diagrammatically summarized in figure 8. Our findings on endosperm patterning in *Acorus* are in general agreement with the earlier interpretation of Buell (1938), who described the chalazal migration of the primary endosperm nucleus and the same early pattern of cellular partitioning.

### *Drimys*

In *Drimys*, the primary endosperm nucleus migrates from an initial position adjacent to the zygote to the chalazal end of the first endosperm cell. After division of the primary en-

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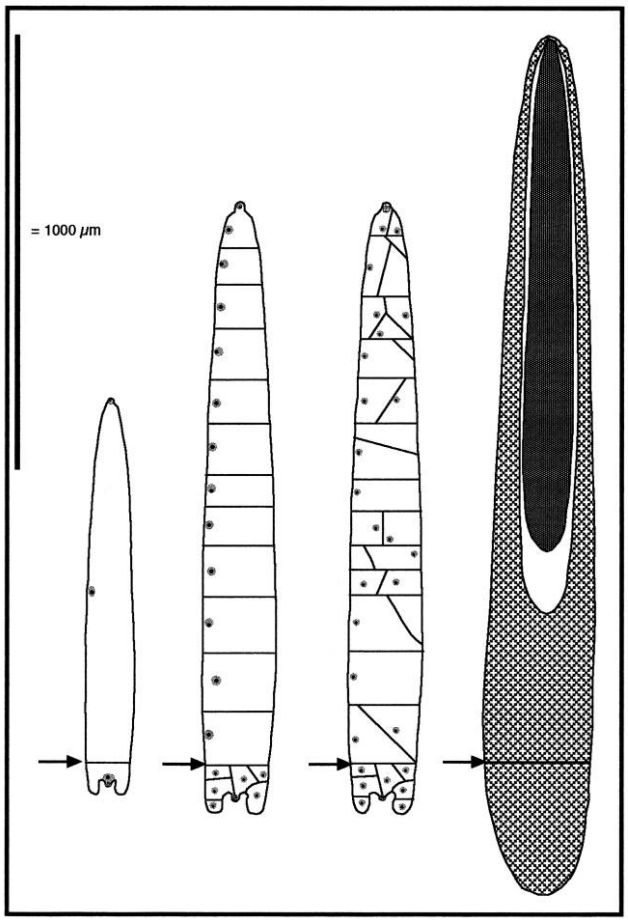


**Fig. 8** Diagrammatic summary of endosperm development in *Acorus calamus* corresponding to stages in fig. 7 and drawn to scale. Arrows indicate the location of the first transverse wall. Nuclei are shown for reference but are not accurately drawn to scale.

dosperm nucleus, a cell wall is formed that partitions the endosperm unequally into two cells (fig. 9*A*). This first partitioning cell wall is usually oblique and separates a large micropylar cell from a smaller chalazal cell (fig. 9*A*). We occasionally observed a nearly transverse wall partitioning the two-celled endosperm. The micropylar cell of the endosperm then divides unequally by means of a transverse wall that partitions the micropylar cell into a small cell at the chalazal end and a large cell at the micropylar end (fig. 9*B*). Another unequal division appears to occur in the larger derivative cell. The new transverse to oblique wall defines a chalazal region consisting of three or four cells (depending on whether or not the chalazal endosperm cell of the two-celled stage has divided yet) and a micropylar region composed of a single large cell (fig. 9*C*).

The first division of the chalazal cell of the two-celled endosperm may be in any plane. Cells in the chalazal domain (consisting of cells derived from both the micropylar cell and the chalazal cell of the two-celled endosperm) divide in numerous planes to produce a multicellular mass of cells that may comprise one half to slightly more of the total endosperm volume (fig. 9*D*). The cells at the extreme chalazal end are smaller and more densely cytoplasmic than other endosperm cells, and the nuclei are always positioned at the chalazal end of the cells.

The micropylar domain (formed exclusively from derivatives of the micropylar cell of the two-celled endosperm) usually divides transversely (once or a few times) to produce a uniseriate region of one, two (usually), or more large vacuolate cells (fig. 9*D*, 9*E*). Differentiated endosperms (with distinct chalazal and micropylar regions) elongate but do not broaden appreciably as the ovule begins to become curved (fig. 9*E*).

Cells of the micropylar domain eventually divide in various planes as the endosperm continues to grow, exhibit more pronounced curvature, and become broader (fig. 9*F*). Endosperm cells remain vacuolate (except for the extreme chalazal cells) as the endosperm enlarges (fig. 9*G*, 9*H*). The endosperm grows to fill the seed as a multicellular tissue composed of numerous cells that are much smaller than in earlier stages (fig. 9*I*). The cells become filled with protein bodies and lipids (fig. 9*J*).

*Drimys* endosperm development is diagrammatically summarized in figure 10. Endosperm development in *Drimys winteri* was reported to be free nuclear by Strasburger (1905). Our findings do not agree with those of Strasburger because we have clearly observed cellular development. Bhandari (1963) described endosperm development in the related plant *Pseudowintera colorata* (also in the Winteraceae). Our observations are quite similar to those aspects of endosperm development reported by Bhandari, who also described a cellular pattern of development, unequal division of the first endosperm cell with either an oblique or transverse wall, and cells at the chalazal end that are small and densely cytoplasmic.

### *Platanus*

The following is a summary of previously published results for *Platanus* (Floyd et al. 1999). After fertilization, the primary endosperm nucleus is located halfway between the micropylar and chalazal ends of the central cell (fig. 11*A*). No permanent cell wall is formed after mitosis of the primary endosperm nucleus, although a transitory cell plate was observed (fig. 11*B*). After several rounds of free nuclear divisions, the endosperm is a coenocyte with a large central vacuole and a parietal layer of cytoplasm and nuclei (fig. 11*C*, 11*D*).

Differentiation of the free nuclear endosperm into two distinct zones results in the aggregation of several nuclei in a common mass of cytoplasm at the chalazal end (the chalazal domain), while the remaining endosperm consists of a thin parietal layer of nuclei around a central vacuole (the micropylar domain) (fig. 11*E*, 11*F*). Cellularization begins in the chalazal domain with a process of simultaneous wall formation that partitions nuclei and cytoplasm into a number of compartments. This process of wall formation is not centripetal. Many of the newly formed chalazal endosperm cells are initially multinucleate; in later developmental stages the chalazal endosperm cells are uninucleate. Cells of the chalazal zone often appear vacuolate just after cellularization (fig. 11*G*). They quickly become densely cytoplasmic.

Following a final wave of mitotic divisions of the free nuclei, the micropylar domain begins the process of cellularization at the chalazal end. Anticlinal walls are formed between nuclei

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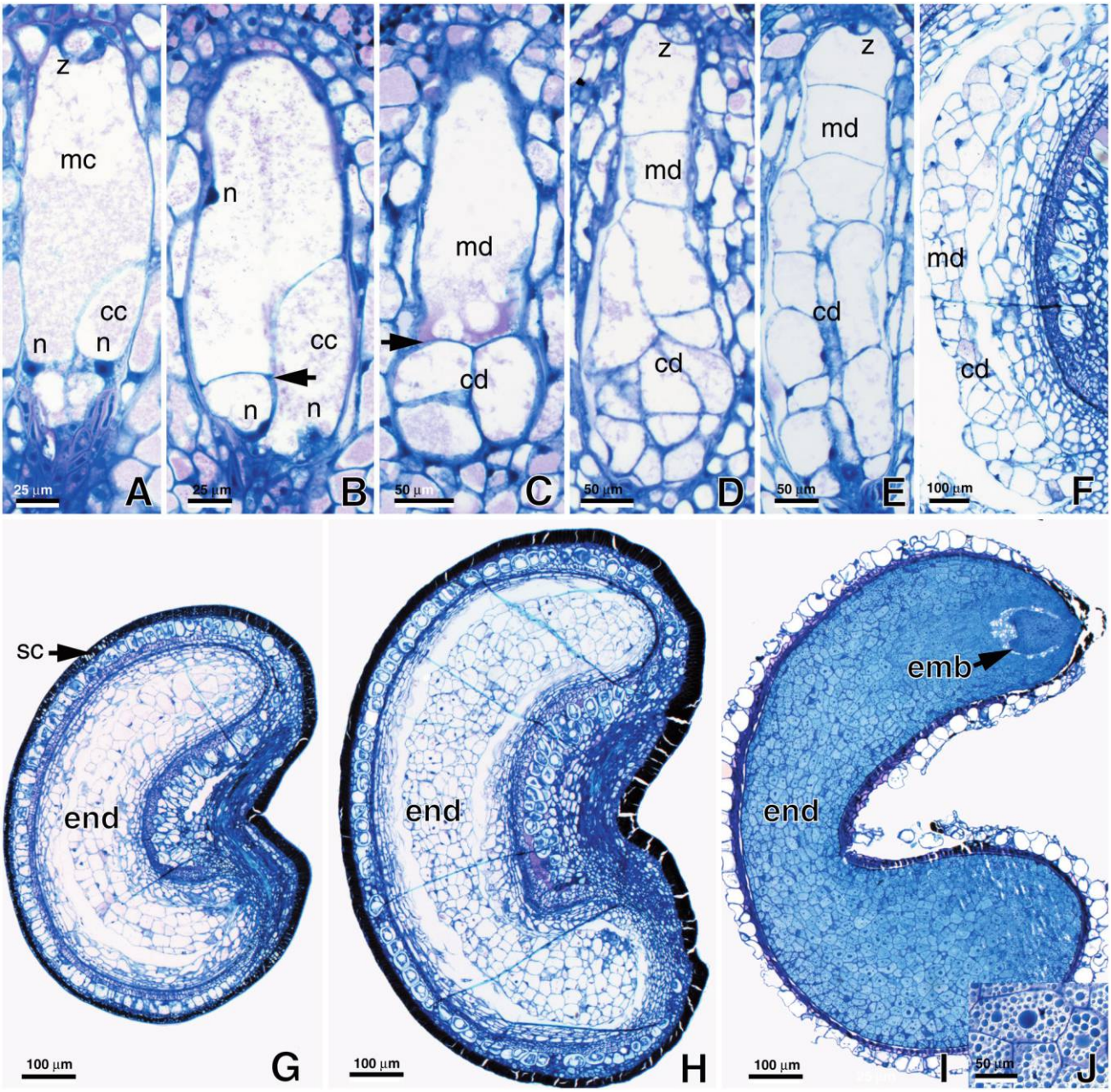


**Fig. 9** Light micrographs showing endosperm development in *Drimys winteri*. All sections are longitudinal and oriented with the micropylar end at the top of the page. *A*, Two-celled endosperm. An oblique wall partitions the endosperm into a small chalazal cell (*cc*) and larger micropylar cell (*mc*). The nuclei (*n*) of both cells are visible at the chalazal end, and the zygote (*z*) remains undivided. *B*, Three-celled endosperm. The micropylar cell has divided with a transverse wall (arrow) at the chalazal end, whereas the chalazal cell (*cc*) remains undivided. All three endosperm nuclei (*n*) are in view. *C*, Four-celled endosperm. Another transverse wall (arrow) has formed, dividing the larger micropylar derivative of the micropylar cell into two cells. The two regions that will exhibit differential development, the micropylar domain (*md*) and the chalazal domain (*cd*), have been established. *D*, Slightly later differentiated endosperm with micropylar domain (*md*), consisting of two large cells in a uniseriate arrangement, and a chalazal domain (*cd*), consisting of many cells in a multiseriate globular arrangement. Zygote (*z*) is still undivided. *E*, Later stage of differentiated endosperm than in *D*. Slight curvature of the endosperm is evident. *F*, Micropylar domain (*md*) is now multiseriate and much longer as the result of endosperm elongation and seed curvature; chalazal domain (*cd*) also has increased in both length and girth. *G*, Entire seed with developing seed coat (*sc*) at slightly later stage than in *F*. Seed curvature more pronounced and endosperm (*end*) both broader and longer. *H*, Slightly later seed than *G*, shown at same scale. Endosperm (*end*) is much broader and longer. *I*, Mature seed with the outer layer of the seed coat removed. Endosperm (*end*) occupies most of the seed volume, and the embryo (*emb*) with cotyledon primordia is shown. *J*, Endosperm tissue at higher magnification. Cells are filled with protein bodies and lipids.

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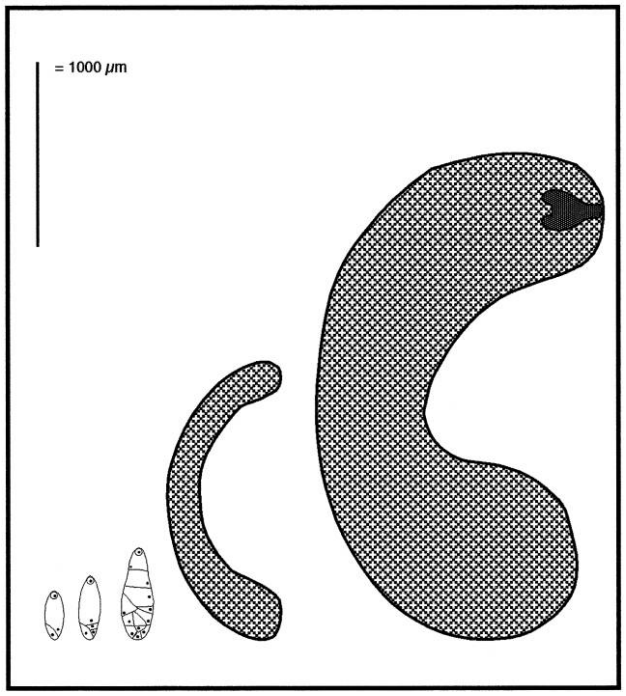


**Fig. 10** Diagrammatic summary of endosperm development in *Drimys winteri*, corresponding to some stages in fig. 9 and drawn to relative scale. Stages represented include fig. 9*A*, 9*B*, 9*D*, 9*F*, and 9*I*. Nuclei are shown for reference but are not accurately drawn to scale.

(fig. 11*G*, 11*H*), which results in a layer of mostly open alveoli surrounding the central vacuole. Cellularization proceeds centripetally in the micropylar zone until it is compartmentalized into uninucleate cells. Continued cell division and development result in clearly differentiated inner and outer endosperm layers within the micropylar zone (fig. 11*I*). The outer endosperm consists of small densely cytoplasmic cells that fill with storage compounds. The inner endosperm remains relatively large-celled and vacuolate. At its peak of development, the endosperm occupies most of the seed volume.

Embryo development is delayed until the earliest stage of endosperm cellularization. The embryo ultimately develops a root apex, shoot apex, and two large cotyledons. As the embryo grows, it replaces the inner endosperm. The large embryo is surrounded by the outer micropylar endosperm and the small amount of chalazal endosperm in the mature seed. Endosperm cells in the mature seed are densely packed with protein bodies and lipids (fig. 11*J*). *Platanus* endosperm development is diagrammatically summarized in figure 12.

## Discussion

### *Comparison of Developmental Patterns among Taxa*

In order to make comparisons of endosperm development across diverse angiosperm taxa, a systematic means of analysis is required. We have identified several discrete stages of endosperm ontogeny in which variable modes of development are expressed among the 13 species investigated.

Endosperm development occurs in two main phases: early pattern formation and later functional specialization. Within the first phase, there are four distinct stages during which a developmental pattern is established; each of these stages can be considered a character with two or more character states (table 2). The first stage, division of the primary endosperm nucleus, may or may not be followed by formation of a cell wall that partitions the first endosperm cell into two cells: the micropylar cell and the chalazal cell. If no cell wall partitions the endosperm at this stage, a free nuclear pattern of development will ensue. If a cell wall is formed after the first mitosis, either a cellular or a helobial developmental pattern may result. Following division of the primary endosperm nucleus (with or without wall formation), the first endosperm cell may be partitioned into two cells or cytoplasmic regions of equal or unequal size. Among endosperms with initial cellular partitioning, development of the micropylar cell may proceed in a cellular or free nuclear manner, and development of the chalazal cell may be cellular, free nuclear, or involve no further proliferation.

Division of the primary endosperm nucleus is cellular in most basal angiosperm taxa (but is free nuclear in *Platanus*). Unequal partitioning of the first endosperm cell into a larger micropylar cell (cytoplasmic region in *Platanus*) was found in most basal taxa that we examined, although partitioning of the first endosperm cell into two equal cells occurs in *Liriodendron* and possibly *Sarcandra*. In *Ceratophyllum* the chalazal cell is larger than the micropylar cell. Development of the micropylar cell is most commonly initially cellular and uniseriate, with exceptions in *Cabomba* and *Platanus*, each of which has free nuclear development in the micropylar cell or micropylar cytoplasmic region, respectively. Finally, development of the chalazal cell is cellular in the majority of basal taxa and often involves early cell divisions in multiple planes. In *Cabomba* and *Saururus* the chalazal cell and nucleus never divide. In *Platanus* development of the chalazal region is free nuclear.

In all basal taxa for which we have data, micropylar and chalazal cells or regions express differential developmental fates, which reflects the bipolar nature of endosperm. This bipolarity involves more extensive development of the micropylar cell or cytoplasmic region to produce most of the mature endosperm and more limited development of the chalazal cell. The bipolar nature of endosperm is also manifest in the independent patterns of development in micropylar and chalazal poles of the same endosperm. These may include different patterns of cell division (e.g., *Acorus*, *Ceratophyllum*, and *Drimys*), different modes of development (e.g., *Cabomba*, with no proliferation of the chalazal cell and free nuclear development of the micropylar cell), or distinct cytoplasmic characteristics and processes of cellularization (e.g., *Platanus*).

The stages and character-state distributions of the later developmental phase are compared in table 3. Total endosperm development may be extensive, moderate, or limited. Endosperm present in the mature seed may be extensive (copious), moderate, limited, or there may be none. "Extensive" total endosperm development indicates that the endosperm (at its maximum) occupies most of the seed volume to the exclusion of other tissues (nucellus and embryo); "moderate" means that substantial endosperm develops but abundant nucellus tissue is also present (when maximum of endosperm development

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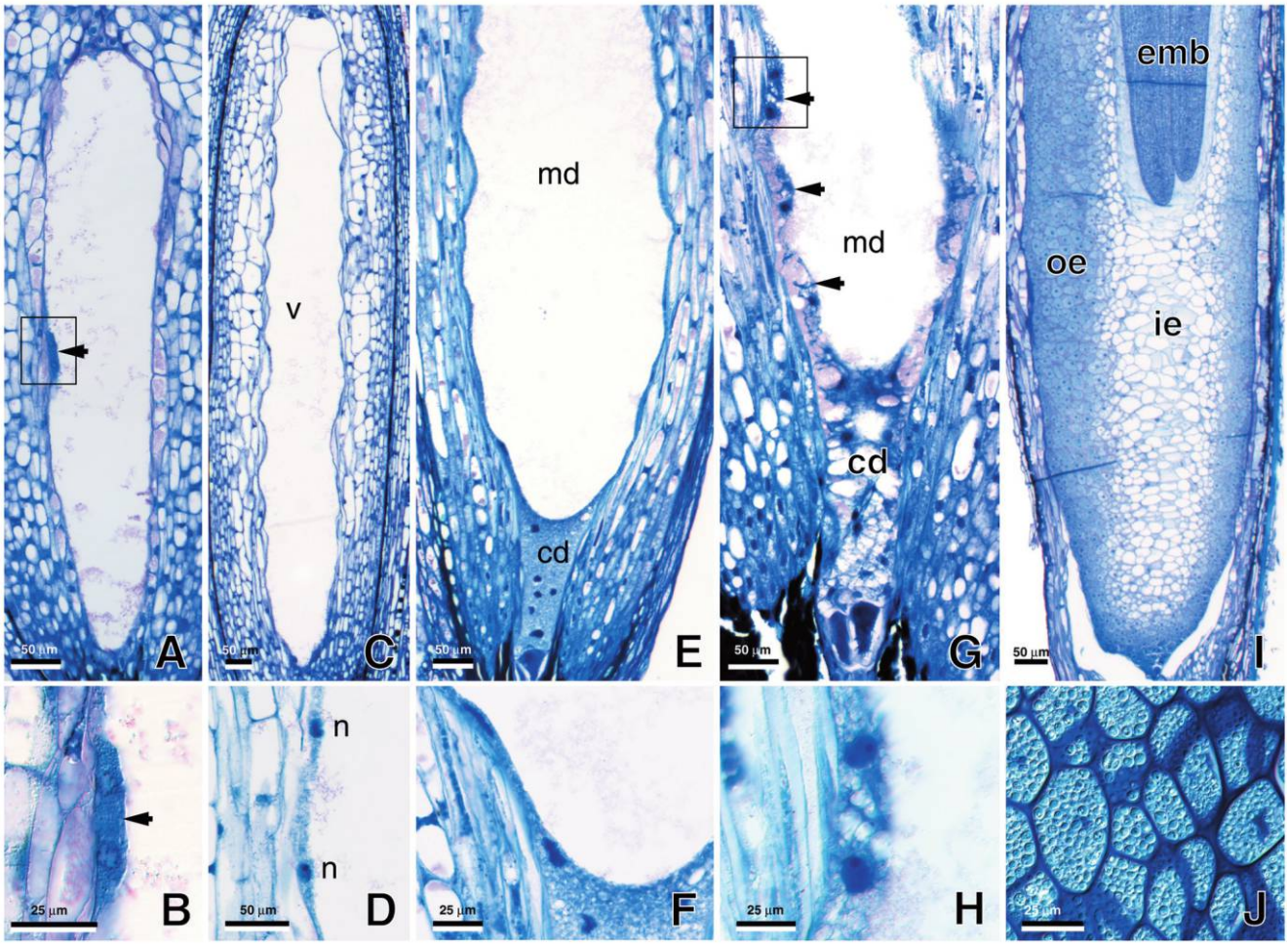


**Fig. 11** Light micrographs showing endosperm development in *Platanus racemosa*. All sections are longitudinal and oriented with the micropylar end at the top of the page. *A*, Division of the primary endosperm nucleus (arrow). *B*, Higher-magnification view of boxed region of *A*; primary endosperm nucleus in telophase. Transitory cell plate is indicated by arrow. *C*, Early free nuclear phase with nuclei distributed in parietal layer of cytoplasm around central vacuole (*v*). *D*, Higher-magnification view of two free nuclei (*n*) in thin layer of cytoplasm from a different serial section of the same endosperm in *C*. *E*, Differentiated free nuclear endosperm, with distinct chalazal domain (*cd*) and micropylar domain (*md*). *F*, Higher-magnification view of transition zone between micropylar domain and chalazal domain from different serial section of same endosperm in *E*. *G*, Early anticlinal wall (arrows) formation of the micropylar domain (*md*). Chalazal domain (*cd*) has already cellularized. *H*, Higher-magnification view of boxed region of *G*; anticlinal wall forming between pair of nuclei. *I*, Completely cellularized endosperm differentiated into inner (*ie*) and outer (*oe*) layers. The embryo (*emb*) has begun to grow into and replace the inner endosperm. *J*, Mature endosperm (*end*) cells filled with protein bodies and lipids.

has been reached), and "limited" describes endosperm that always occupies a much smaller volume of the seed relative to the nucellus. Separate from the degree of total endosperm development, it is possible to characterize the amount of endosperm in the mature seed. This may vary from extensive (copious) to moderate, limited, or none. With respect to storage compounds, endosperm tissue may accumulate proteins and lipids, lipids only, or lack significant nutritive reserves. Starch was never detected in the endosperms of any of the basal angiosperms that we studied. Finally, the presence (or absence) and type of nonendosperm storage tissue present in the seed is also variable among basal angiosperms (table 3).

Two of the later functional characters are nearly invariant among the 13 basal angiosperm taxa. Total endosperm production is extensive in most, but limited in *Cabomba* and *Saururus*, and moderate in *Acorus*. Storage compounds are a combination of proteins and lipids in most taxa. However, only lipids occur in *Cabomba* endosperm. In *Ceratophyllum* and *Calycanthus*, no storage compounds accumulate in the endosperm (both taxa lack appreciable endosperm in the mature seed). The presence of protein bodies in the endosperm of basal angiosperms has been largely overlooked by embryologists (Johri et al. 1992 and references therein). The third aspect of endosperm structure and function, endosperm in the mature seed, is most commonly extensive. However, *Acorus* and *Platanus* seeds retain only a moderate amount of endosperm. Little endosperm is present in the mature seeds of *Cabomba* and *Saururus*, and there is no appreciable endosperm in mature

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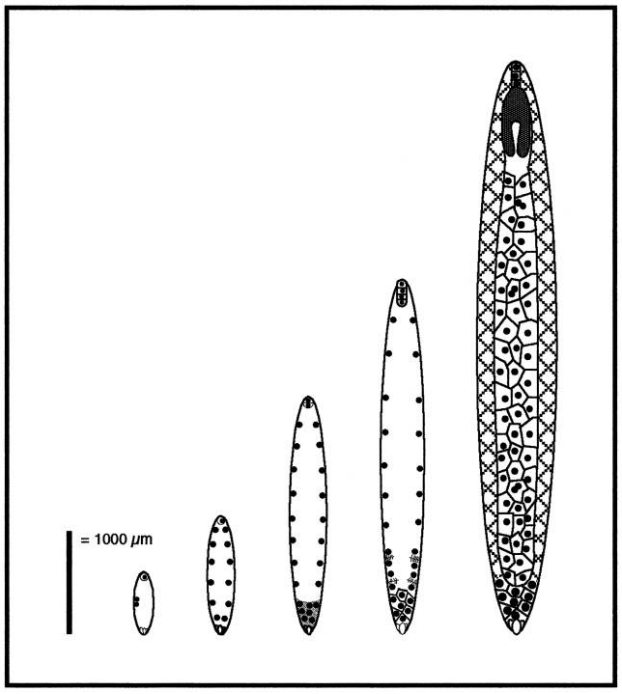


**Fig. 12** Diagrammatic summary of endosperm development in *Platanus racemosa* corresponding to stages in fig. 11 and drawn to relative scale. Nuclei are shown for reference but are not accurately drawn to scale.

seeds of *Ceratophyllum* and *Calycanthus*. Finally, in seven (of 13) taxa surveyed, endosperm is the sole storage tissue in the seed. *Acorus*, *Cabomba*, and *Saururus* have a nucellus-derived accessory storage tissue, whereas *Acorus*, *Ceratophyllum*, *Platanus*, and *Calycanthus* store food reserves in the embryo.

### *Ancestral Endosperm and Evolution of Early Developmental Characters*

The preceding comparative analysis indicates that many features of endosperm development are common or nearly invariant among basal angiosperms, despite the widely varying patterns of development observed (e.g., cellular, free nuclear, helobial). Parsimonious character optimization onto the recently published phylogenies of Qiu et al. (1999) and Soltis et al. (1999) resolves the most commonly occurring states among the basal taxa in this study as plesiomorphic for angiosperms in all cases (tables 2, 3). This indicates that primitive endosperm development involves the formation of a cell wall after division of the primary endosperm nucleus. This initial cell division unequally partitions the first endosperm cell into a larger micropylar cell that will then undergo cellular uniseriate development and a smaller chalazal cell that will initiate a cellular pattern of development. Plesiomorphic endosperm appears to involve differential developmental fates of chalazal and micropylar regions (bipolar development). During the later functional phase, total endosperm production is extensive, and this copious endosperm is retained in the mature seed. Most of the endosperm appears to originate from derivatives of the micropylar cell of the two-celled endosperm. Among basal angiosperms, endosperm as the sole storage tissue, provisioned with proteins and lipids, is resolved as plesiomorphic (table 4).

The analysis of endosperm development as a series of discrete stages allows for more than the determination of a suite of plesiomorphic character states. This approach also provides insight into how endosperm patterns are developmentally established and evolutionarily transformed. The basic pattern of endosperm development is initiated during division of the primary endosperm nucleus and first endosperm cell, and subsequent development in the micropylar and chalazal cells or regions. Parsimonious character optimization clearly resolves formation of a cell wall following division of the primary endosperm nucleus as plesiomorphic (fig. 13), and this early developmental event was retained throughout the magnoliids, in the monocot common ancestor (and *Acorus*), and in the eudicot common ancestor. A free nuclear first division evolved independently within the monocots and several times in the basal eudicots (as is manifest in *Platanus*) (Floyd et al. 1999).

Early development of the micropylar cell or cytoplasmic region is either cellular and uniseriate or free nuclear among basal angiosperms (table 2). Optimization of micropylar development (coded as either cellular or free nuclear) (fig. 14) clearly resolves cellular development as plesiomorphic. This condition was retained in the monocot common ancestor (and *Acorus*). A transformation from cellular to free nuclear micropylar development occurred in Cabombaceae (a helobial type of endosperm), and this transformation has also occurred independently in the lower monocots and among lower eudicots.

Finally, development of the chalazal cell or cytoplasmic region, coded as cellular, free nuclear, or none (indicating no nuclear or cellular division), was parsimoniously optimized (fig. 15). Once again, cellular was clearly resolved as plesiomorphic for angiosperms, and this primitive condition was retained in the monocot common ancestor. Free nuclear chalazal development evolved independently within the monocots and lower eudicots, and lack of nuclear or cellular proliferation (none) evolved twice: in the common ancestor of the Nymphaeales and in *Saururus*. Although not clearly shown in figure 15, this character state (lack of nuclear or cellular proliferation) also occurs in the Araceae (Grayum 1991), indicating a third independent origin within the monocot clade.

It is evident, based on the three preceding optimization analyses, that division of the primary endosperm nucleus, development of the micropylar cell or region, and development of the chalazal cell or region have evolved independently in many cases (fig. 16). Different combinations of character states for division of the primary endosperm nucleus, development in the micropylar cell or region, and development of the chalazal cell or region have led to the establishment of not three basic patterns (free nuclear, cellular, and helobial) but at least six patterns among magnoliids, basal monocots, and basal eudicots (fig. 17; table 3). There are no cases in which free nuclear division of the primary endosperm nucleus is immediately followed by cellular development of micropylar and chalazal regions (although in *Platanus*, cellular development ultimately occurs throughout the endosperm). Thus, there is only one pattern or combination of early developmental characters associated with free nuclear division of the primary endosperm

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**Table 2**

**Expression of Three Endosperm Developmental Characters Associated with Early Pattern Formation and the Presence of Differential Developmental in Micropylar and Chalazal Regions in 13 Basal Angiosperm Taxa**

| Taxa | PEN first division | Partitioning of first endosperm cell | Early micropylar development | Early chalazal development | Subsequent development |
|---|---|---|---|---|---|
| *Acorus calamus* ..... | **Cellular** | **Unequal** | **Cellular, uniseriate** | **Cellular** (multiple planes) | **Bipolar** |
| *Cabomba caroliniana* ..... | **Cellular** | **Unequal** | Free nuclear | No division | **Bipolar** |
| *Ceratophyllum demersum* ..... | **Cellular** | **Unequal** | **Cellular, uniseriate** | **Cellular** (one transverse division) | **Bipolar** |
| *Platanus racemosa* ..... | Free nuclear | **Unequal** | Free nuclear | Free nuclear | **Bipolar** |
| *Illicium mexicanum*, *Illicium floridanum* ..... | **Cellular** | **Unequal** | **Cellular, uniseriate** | **Cellular** (multiple planes) | **Bipolar** |
| *Drimys winteri* ..... | **Cellular** | **Unequal** | **Cellular, uniseriate** | **Cellular** (multiple planes) | **Bipolar** |
| *Sarcandra glabra*, *Sarcandra chloranthoides* ..... | **Cellular** | Equal | **Cellular** | **Cellular** | ? |
| *Liriodendron tulipifera* ..... | **Cellular** | Equal | **Cellular, uniseriate** | **Cellular** | ? |
| *Saururus cernuus* ..... | **Cellular** | **Unequal** | **Cellular, uniseriate** | No division | **Bipolar** |
| *Amborella trichopoda* ..... | **Cellular** | **Unequal** | **Cellular** | **Cellular** (multiple planes) | **Bipolar** |

Note. Question marks indicate missing data. Data in bold represent putative plesiomorphic character states.

nucleus. In contrast, following cellular division of the primary endosperm nucleus, we have identified five additional patterns of endosperm that involve independent development of the chalazal cell and the micropylar cell (fig. 17; table 3). These five patterns include cellular development of both chalazal and micropylar cells (e.g., *Ceratophyllum*), free nuclear development in both chalazal and micropylar cells (e.g., *Platanus*), free nuclear development of the micropylar cell and cellular development in the chalazal cell (e.g., some members of the Araceae), free nuclear development of the micropylar cell and no proliferation of the chalazal cell (e.g., *Cabomba*), and finally, cellular development of the micropylar cell and no further proliferation of the chalazal cell (e.g., *Saururus*). Identification of the independently evolving features that establish developmental patterns allows for the recognition of the true diversity of ontogenies and can serve as a basis for understanding how different patterns may have evolved.

### *Differential Developmental Fate of Chalazal and Micropylar Domains*

It is clear from the preceding discussion that there is significantly more complexity in early endosperm development than can be described with reference to the typological system of "cellular," "free nuclear," and "helobial." Paradoxically, there is also an underlying commonality to all endosperms. Despite differences in basic pattern, one aspect of early development may be shared by all basal angiosperm taxa: differential developmental fates of the chalazal and micropylar regions of the endosperm (table 2). This bipolarity is expressed regardless of the presence or absence of cell walls. *Platanus*, the single free nuclear taxon in this study, exhibits differentiation into chalazal and micropylar regions, as do the many cellular taxa (e.g., *Amborella* and *Acorus*) and helobial *Cabomba*, which has free nuclear development in the micropylar cell.

The polar regions that express distinct patterns of development may or may not be precisely defined by an initial cellular partition (fig. 18). In *Ceratophyllum*, a single derivative cell of the initial micropylar cell develops into a globular micropylar mass, whereas the remaining micropylar cell derivatives develop, along with chalazal cell derivatives, into a uniseriate vacuolate chalazal region (fig. 3). In both *Cabomba* (helobial development) and *Acorus* (cellular development), the initial cellular division corresponds directly to the demarcation of the two regions that subsequently express independent developmental fates (figs. 5, 7). Finally, *Platanus* lacks a partitioning wall after division of the primary endosperm nucleus but still exhibits differential development of the two poles, as can be seen in the distinctly different organizations of free nuclear cytoplasm and methods of cellularization. In addition, the chalazal and micropylar regions of *Platanus* endosperm contribute unequally to the mature endosperm (fig. 11).

To properly account for the bipolar nature of endosperm and differential developmental potential of the two poles, we refer to the differentially developing regions as the "chalazal domain" and the "micropylar domain." This avoids the use of terminology associated with any particular developmental pattern (e.g., cells or chambers). In all of the basal angiosperms examined (for which were able to obtain data), it is the micropylar domain that contributes the most to the mature endosperm tissue (except perhaps in *Drimys*), whereas the chalazal domain exhibits more limited developmental potential. In

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**Table 3**

**Expression of Four Characters Associated with the Later Phases of Endosperm Development in 13 Basal Angiosperm Taxa**

| Taxa | Total endosperm development | Endosperm in mature seed | Endosperm storage | Accessory storage tissue |
|---|---|---|---|---|
| *Acorus calamus* ..... | Moderate | Moderate (large embryo) | **Protein, lipid** | Present, nucellus |
| *Cabomba caroliniana* ..... | Limited | Little (large embryo) | Lipid | Present, nucellus (starch) |
| *Ceratophyllum demersum* ..... | **Extensive** | None (large embryo) | None | Present, embryo (starch) |
| *Platanus racemosa* ..... | **Extensive** | Moderate (large embryo) | **Protein, lipid** | Present, embryo (protein/oil) |
| *Illicium mexicanum*, *Illicium floridanum* ..... | **Extensive** | **Extensive** (small embryo) | **Lipid, protein** | **None** |
| *Drimys winteri* ..... | **Extensive** | **Extensive** (small embryo) | **Lipid, protein** | **None** |
| *Sarcandra glabra*, *Sarcandra chloranthoides* ..... | **Extensive** | **Extensive** (small embryo) | **Lipid, protein** | **None** |
| *Calycanthus floridus* ..... | **Extensive** | None (large embryo) | None | Present, embryo (protein, lipid, starch) |
| *Liriodendron tulipifera* ..... | **Extensive** | **Extensive** | **Lipid, protein** | **None** |
| *Saururus cernuus* ..... | Limited | Little | **Lipid, protein** | Present, nucellus (starch, protein) |
| *Amborella trichopoda* ..... | **Extensive** | **Extensive** | **Protein, lipid** | **None** |
| *Austrobaileya scandens* ..... | **Extensive** | **Extensive** | **Lipid, protein** | **None** |
| *Schisandra sphenanthera* ..... | **Extensive** | **Extensive** | **Protein, lipid** | **None** |

Note. Data in bold represent putative plesiomorphic character states.

all cases, the mature endosperm tissue is ultimately derived primarily from the micropylar cell of the two-celled endosperm (or micropylar region in *Platanus*).

The expression of differing developmental fates of micropylar and chalazal domains of the endosperm is not unique to basal angiosperm lineages. Micropylar/chalazal endosperm polarity is also known from endosperms of more recently derived monocot and eudicot taxa including *Zea*, *Arabidopsis*, *Capsella* (and other Brassicaceae), *Glycine*, and *Helianthus* (Brink and Cooper 1947; Newcomb 1973; Schulz and Jensen 1974; Prabhakar 1979; Mansfield and Briarty 1990; Olsen et al. 1992; Chamberlin et al. 1994; Kranz et al. 1998; Berger 1999; Brown et al. 1999). Differential patterns of gene expression in micropylar and chalazal regions have also been discovered in developing maize endosperm (Hueros et al. 1995; Doan et al. 1996; Opsahl-Ferstad et al. 1997; Olsen 1998). Some authors have attributed these differences to environmental stimuli within the ovule, such as the influence of the chalazal nucellus and the embryo (Schulz and Jensen 1974; Prabhakar 1979; Vijayaraghavan and Prabhakar 1984).

**Table 4**

**Hypothesized Plesiomorphic Features of Endosperm**

1. Differential development of micropylar and chalazal domains
2. Formation of a cell wall after division of primary endosperm nucleus
3. Unequal division of the central cell to form a larger initial micropylar cell
4. Cellular, uniseriate development of micropylar domain
5. Cellular development of the chalazal domain
6. Extensive total endosperm production
7. Copious endosperm in the mature seed
8. Endosperm as the sole storage tissue
9. Proteins and lipids as endosperm primary storage compounds

Kranz et al. (1998) found that maize endosperms formed and grown *in vitro* (i.e., removed from any influence of ovule or embryo) exhibit marked polar differentiation. *In vitro* maize endosperms characteristically develop a constriction that defines a smaller "globular" region and a longer "oblong" region. Development in both regions is initially free nuclear, but following cellularization, the globular region comprises small densely cytoplasmic cells, whereas the oblong region consists of larger more vacuolate cells. These recent findings in cellular and molecular biology indicate that intrinsic morphogenetic factors may be involved in the patterning of endosperm in chalazal and micropylar poles or domains. Furthermore, development of *in vitro* maize endosperms is strikingly similar to stages we observed in basal angiosperms, particularly *Cabomba* and *Ceratophyllum* (figs. 3*C*, 4, 5*E*, 6). Both of these basal taxa exhibit an endosperm composed of a small globular region that is more densely cytoplasmic and a larger elongate region that is more highly vacuolate. It is clear that there are striking similarities in endosperm organization in these distantly related angiosperms (two magnoliids and a derived

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monocot) (fig. 2), and the basic pattern of differential bipolar development may be shared with other distantly related higher eudicot lineages mentioned above (Brassicaceae, Fabaceae, Asteraceae). The occurrence of this putatively plesiomorphic feature (differential development of micropylar and chalazal domains) in such distant relatives may indicate that this is a ubiquitous conserved fundamental feature of endosperm development.

### *Evolution of Later Functional Endosperm Characters*

Evolutionary changes have also occurred in the functional specialization characters associated with later stages of endosperm development. These include total endosperm development, the amount of endosperm in the mature seed, the nature of storage compounds, and the role of endosperm in seed storage. A reduction of endosperm in the mature seed (from the plesiomorphic extensive condition) is characteristic of several basal angiosperm taxa (*Acorus*, *Cabomba*, *Ceratophyllum*, *Platanus*, *Calycanthus*, *Saururus*) (table 3). Parsimonious optimization onto the topology of Qiu et al. (1999) indicates that each of these taxa represents a separate evolutionary origin of reduced endosperm (fig. 19). It appears that reductions in endosperm in the seed among basal angiosperms are always coupled with the presence/acquisition of other storage tissues, either the embryo or nucellus (fig. 19; table 3). Reduction in endosperm (in the seed), accompanied by an increase in embryo size, has been described as an evolutionary trend in advanced angiosperm groups (Martin 1946; Stebbins 1974; Cronquist 1988; Takhtajan 1991) but has not been explicitly recognized as common among basal lineages.

Modifications in the tissues involved in seed storage seem to have evolved via different evolutionary pathways (Johnson 1902; Coulter and Chamberlain 1903). There are at least four modes of development that differ from the plesiomorphic pattern among basal angiosperms. First, the endosperm may develop extensively, as in plesiomorphic angiosperms, and then be partially consumed by the embryo during seed maturation (*Platanus*). Second, the endosperm may undergo extensive growth in volume accompanied by limited cellular development, only to be displaced by the embryo (*Ceratophyllum*). A third derived pattern involves development of substantial endosperm storage tissue, accompanied by formation of an additional nucellus-derived tissue (*Acorus*). Finally, in *Cabomba* and *Saururus*, essentially all storage function has been assumed by nucellar tissue (which occupies most of the mature seed), and the endosperm undergoes only limited development and growth (but is present in the mature seed). It should be noted that the presence of a nucellar-derived storage tissue (perisperm) in each of the aforementioned taxa represents an independent evolutionary origin that is reflected in major differences in the initiation and development of perisperm tissue in representative taxa such as *Acorus*, *Cabomba*, and *Saururus* (Rudall 1997; Rudall and Furness 1997). Among basal angiosperms, a reduction in endosperm development is always associated with development of a nonendosperm nonembryo storage tissue. Extensive development of endosperm followed by a reduction during seed maturation appears to always be associated with the assumption of a storage role by the embryo.

There have also been evolutionary changes in the type of

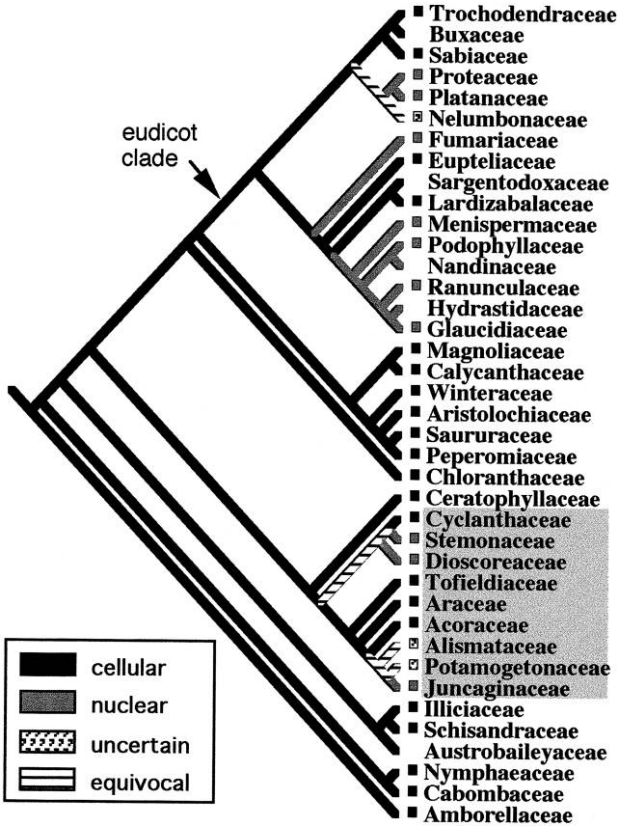


**Fig. 13** Division of the primary endosperm nucleus, coded as either cellular or free nuclear, parsimoniously optimized onto an angiosperm phylogeny based on Qiu et al. (1999). Shaded box indicates the monocot clade. Cellular division is resolved as plesiomorphic for angiosperms and is retained throughout the magnoliids in the monocot common ancestor and *Acorus* and in the eudicot common ancestor. Free nuclear division has evolved independently in the monocots and has evolved independently several times within the lower eudicots. Optimization onto the phylogeny of Soltis et al. (1999) leads to the same conclusions. Data for taxa not included in this study are from Cook (1902, 1906, 1909), Mabberley (1987), Grayum (1991), and Johri et al. (1992).

seed storage compounds found among basal lineages of flowering plants. Starch, rather than the plesiomorphic proteins and lipids, is the predominant seed storage compound for *Ceratophyllum*, *Cabomba*, and *Saururus*. However, starch is stored in the embryo in *Ceratophyllum* and in the nucellus in *Cabomba* and *Saururus*. No starch was observed in the endosperms of any of the taxa observed in this study. The small amount of endosperm in the seed of *Saururus* contains lipids and proteins. Similarly, the limited endosperm in the *Cabomba* seed accumulates lipids, and there are small protein bodies present in the endosperm cytoplasm during development in both *Ceratophyllum* and *Cabomba*. Thus, all three taxa appear to have retained at least some vestige of the plesiomorphic endosperm storage compounds (proteins and lipids), whereas the novel storage compound, starch, accumulates in an alternate tissue (embryo or nucellus). This indicates a developmental constraint (*sensu* Alberch 1981) on the accretion of

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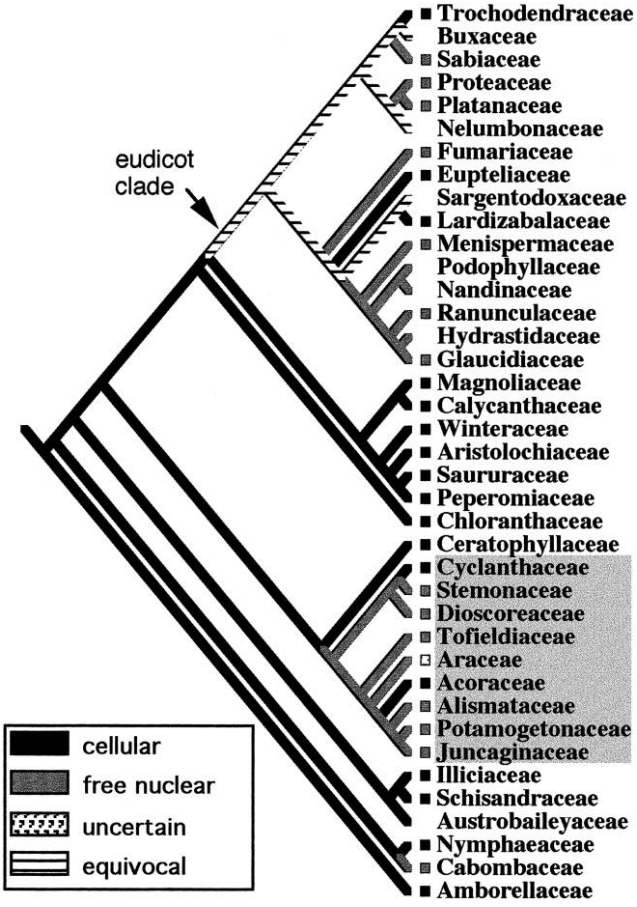


**Fig. 14** Micropylar development, coded as either cellular or free nuclear, parsimoniously optimized onto an angiosperm phylogeny based on Qiu et al. (1999). Shaded box indicates the monocot clade. Cellular development is resolved as plesiomorphic for angiosperms and is retained throughout the magnoliids and in the monocot common ancestor. The plesiomorphic condition for eudicots is equivocal. Free nuclear development has evolved independently in Cabombaceae, in the monocots, and in the lower eudicots. Optimization onto the phylogeny of Soltis et al. (1999) leads to the same conclusions. Data for taxa not included in this study are from Cook (1902, 1906, 1909), Mabberley (1987), Grayum (1991), and Johri et al. (1992).

storage reserves in endosperm during the early radiation of angiosperms. Given the importance of starchy endosperm to human nutrition (in the form of cereal grains) (Esau 1977; Larkins and Vasil 1997), an obvious and important question that arises from the foregoing discussion is, When and how did flowering plants acquire the ability to store abundant starch reserves in the endosperm?

### *Endosperm Evolution among Basal Angiosperms: General Patterns*

Having discussed both the early pattern-forming phase and the later functional specialization phase of endosperm development in some detail, we can now examine the broader evolutionary patterns for basal angiosperms. Despite the widespread occurrence of plesiomorphic endosperm features among basal angiosperms, only three taxa, *Amborella*, *Illicium*, and *Drimys*, exhibit all nine of the primitive character states that we have identified (table 2; plesiomorphic states indicated by bold type). By dissecting endosperm development into natural developmental components and tracing the phylogenetic history of each character, the assessment of an ancestral ontogenetic pattern for endosperm has been achieved. It has been asserted that "the extant archaic angiosperms are a heterogeneous assemblage from which the derivation of an accurate assessment of plesiomorphic traits is unlikely" (Chase 1998, p. 2). Our analysis reveals that nothing could be further from the truth.

Parsimonious optimization indicates that magnoliids have mostly retained plesiomorphic aspects of early endosperm pattern formation and thus exhibit the primitive bipolar cellular

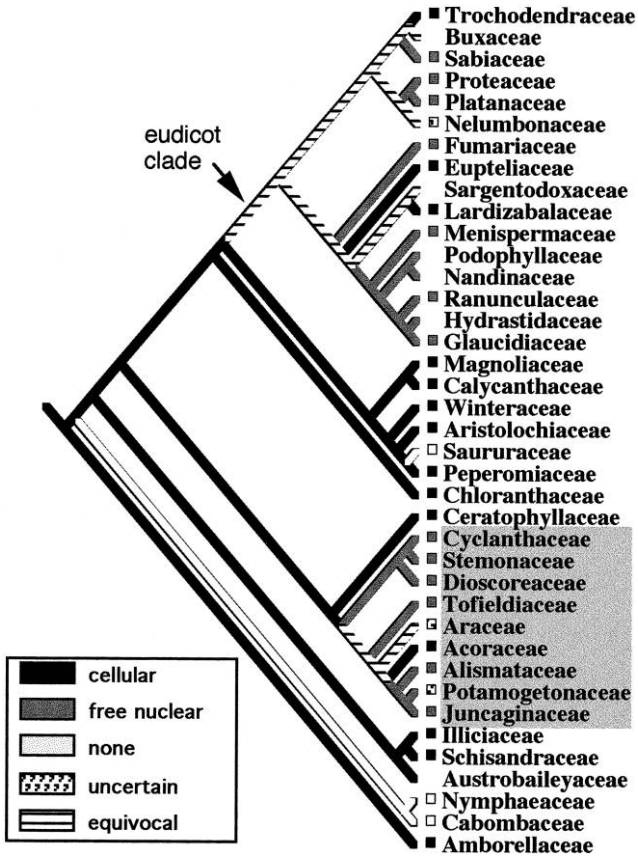


**Fig. 15** Chalazal development, coded as cellular, free nuclear, or none (indicating no nuclear or cellular division), parsimoniously optimized onto an angiosperm phylogeny based on Qiu et al. (1999). Shaded box indicates the monocot clade. Cellular development is resolved as plesiomorphic for angiosperms and is retained throughout the magnoliids and in the monocot common ancestor. The plesiomorphic condition for eudicots is equivocal. Free nuclear development has evolved independently in the monocots and has evolved independently within the lower eudicots. No further division has evolved independently in the ancestor of the Nymphaeales and Saururaceae. Optimization onto the phylogeny of Soltis et al. (1999) leads to the same conclusions. Data for taxa not included in this study are from Cook (1902, 1906, 1909), Mabberley (1987), Grayum (1991), and Johri et al. (1992).

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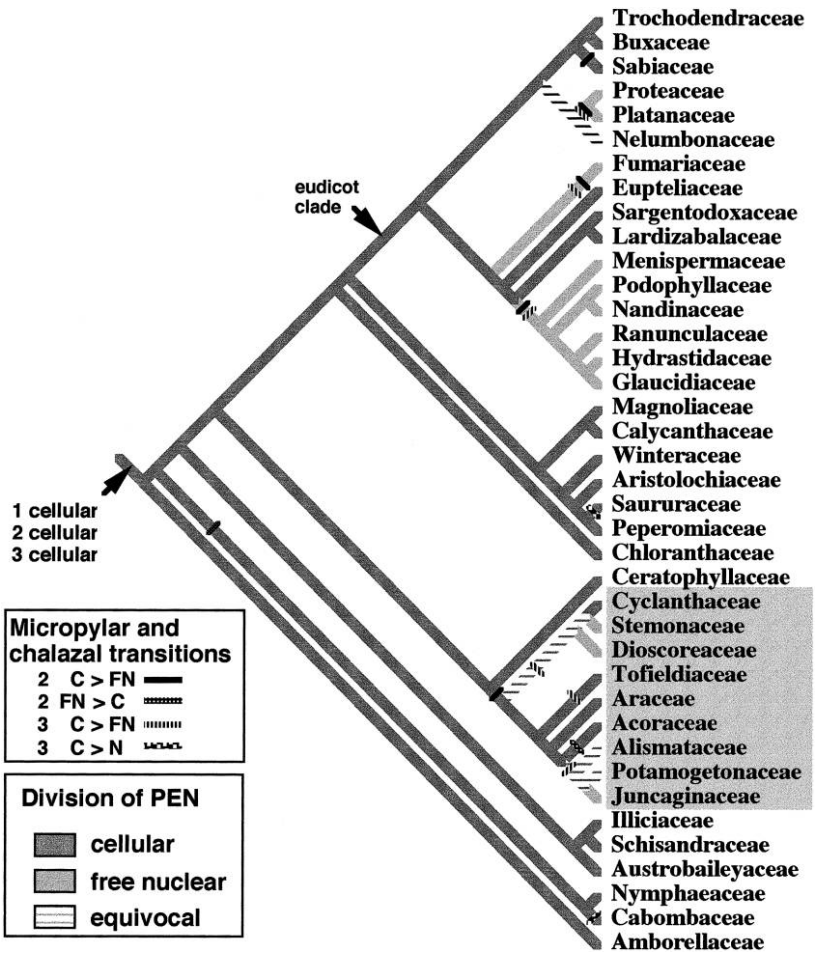


**Fig. 16** Three early pattern-forming characters, (1) division of the primary endosperm nucleus (*PEN*), (2) micropylar development, and (3) chalazal development, mapped onto the phylogeny of Qiu et al. (1999). Shaded branches show character states for division of the PEN; tick marks indicated transitions of characters *2* and *3*. Character *2* has transitioned from plesiomorphic cellular (*C*) to free nuclear (*FN*) and from free nuclear back to cellular; character *3* has changed from cellular to free nuclear and to no further development (*N*). Shaded box indicates the monocot clade. Data for taxa not included in this study are from Cook (1902, 1906, 1909), Mabberley (1987), Grayum (1991), and Johri et al. (1992).

ontogeny (with a few exceptions). Numerous modifications of these developmental characters occurred early in monocot and eudicot diversification (figs. 13–15; table 2). Similarly, most aspects of endosperm functional specialization have been conserved in magnoliid lineages. Endosperm development is typically extensive, and storage compounds in the form of proteins and lipids are found in the endosperms of most magnoliids (table 3). There is more variability in the amount of endosperm in the mature seed (which came about through a variety of processes; see discussion above) and in the presence and type of accessory storage tissue (either embryo or nucellus) (fig. 19; table 3). Together, these data indicate that endosperm ontogeny was highly conserved throughout the radiation of magnoliid angiosperms, except within in a few lineages: Cabombaceae, *Saururus*, *Calycanthus*, Lauraceae (Johri et al. 1992; Heo et al. 1998), and *Piper* (Johri et al. 1992).

The general conformity in distribution of endosperm developmental characters stands in contrast to some other angiosperm reproductive characters, such as floral organization (Endress 1987*b*) and pollen structure (Sampson 2000), which are highly variable among basal flowering plants. The apparent diversification of endosperm developmental patterns appears to coincide with the early radiation of certain more highly nested clades, particularly eudicots and monocots (figs. 13–15, 19; table 5) and may thus reflect independent evolutionary experimentation and modification of reproductive biology during the early history of these two major angiosperm lineages. It has been suggested that increased rates of diversification (biological and speciation) occurred not with the origin of the flowering plant clade but later during angiosperm diversification (Drinnan et al. 1994; Sanderson and Donoghue 1994; Mathews and Donoghue 1999). Shifts in evolutionary patterns of endosperm character diversification may reflect shifts in patterns of taxon diversification. However, data are lacking for many taxa. A better understanding of endosperm development in additional basal monocot and eudicot taxa will be informative in reconstructing the early evolution of endosperm in

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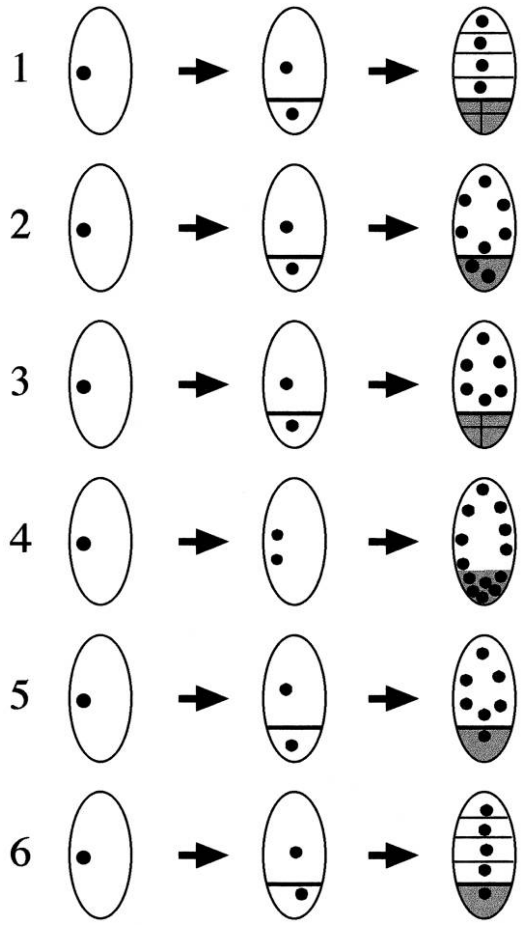


**Fig. 17** Six different basic endosperm developmental patterns identified, based on the results presented in this article and from the literature, corresponding to table 5. Shaded area highlights the chalazal region.

these two major groups of flowering plants and will allow for more robust analyses of patterns of character diversification.

The recognition of an early pattern-formation phase and a later functional specialization phase of endosperm development reflects not only a temporal division but distinct patterns of evolutionary modification as well. It is clear that the many components of early pattern formation in endosperm have evolved and diversified independently. At the very least, six different early ontogenetic patterns of endosperm can be identified (tables 2, 5; fig. 17). In contrast, many features of later endosperm development (table 3) (i.e., the extent of endosperm development and its role in seed storage) appear to have evolved in a correlate fashion with other seed components (embryo and nucellus). This is most certainly related to the functional constraint of seed biology, i.e., the need to produce and provision the next sporophyte generation (embryo) (Brink and Cooper 1947; Esau 1977). Evolutionary trends in the relative size of embryo and endosperm in angiosperm seeds have been discussed mainly within the context of embryo morphology and seed dormancy (Martin 1946; Grushvitsky 1967; Nikolaeva 1999), yet rarely from the perspective of endosperm developmental evolution. Our analysis indicates that all seed tissues must be considered if we are to understand evolutionary change in any component that is involved with seed storage function. Data for additional basal angiosperm taxa, as well as robust phylogenies for lineages other than the three basal angiosperm clades, will aid tremendously in the further assessment of evolutionary trends in aspects of seed storage, including endosperm development. These later aspects of endosperm functional specialization hold tremendous promise for the study of coordinated developmental evolution among the various tissues and organisms that constitute a seed.

### *Potential Significance of Bipolar Endosperm Development*

An intriguing aspect of the identification of differential development of micropylar and chalazal domains is that this pattern is shared not only with other endosperms but with seed plant embryos. Many features of early embryogenesis in angiosperms are similar to the plesiomorphic pattern of endosperm development described herein. These include polarization and unequal division of the initial cell, an early filamentous phase, distinct modes of development in micropylar and chalazal poles with differential contributions to the mature structure, and histogenesis (Goldberg et al. 1994; Kaplan and Cooke 1997; Floyd et al. 1999). Like embryos, differentiation and development of chalazal and micropylar domains are not necessarily precisely defined by cell division. Although endosperm is a determinate structure whose development is limited by seed maturation (DeMason 1997), while embryos are indeterminate and initiate phases of organogenesis and subsequent growth that will continue after the maturation of the seed (Kaplan and Cooke 1997), early developmental stages in endosperms and embryos of basal angiosperms are remarkably similar.

The observation of "embryo-like" qualities in the endosperms of basal angiosperms may have important implications for addressing the question of the evolutionary origin of endosperm. Early in the twentieth century, two competing hypotheses for the evolutionary origin of endosperm were articulated (Friedman, in press). Sargant (1900) proposed that endosperm represents a highly modified supernumerary embryo. Strasburger (1900) and Coulter (1911) both favored an alternative hypothesis that endosperm represents a modified female gametophyte whose development is stimulated by a second fertilization event. In recent years, genetic, developmental, and phylogenetic evidence has been presented that supports the hypothesis that endosperm may have been derived from a supernumerary embryo (Haig and Westoby 1989*a*, 1989*b*; Queller 1989; Friedman 1990, 1992*a*, 1992*b*, 1994, 1995). In contrast, no evidence has been presented that is congruent with the "gametophyte-origin" hypothesis (Friedman, in press). Endosperm developmental patterning in basal angiosperms is quite unlike female gametophyte developmental patterning in most nonflowering seed plants, which begins with an extensive free nuclear phase followed by a distinct process of alveolarization (Maheshwari and Singh 1967; Gifford and Foster 1989; Friedman and Carmichael 1998), or in flowering plants, which usually entails three free nuclear divisions followed by incomplete cellularization (Gifford and Foster 1989). There are no reports of unequal cytoplasmic partitioning or

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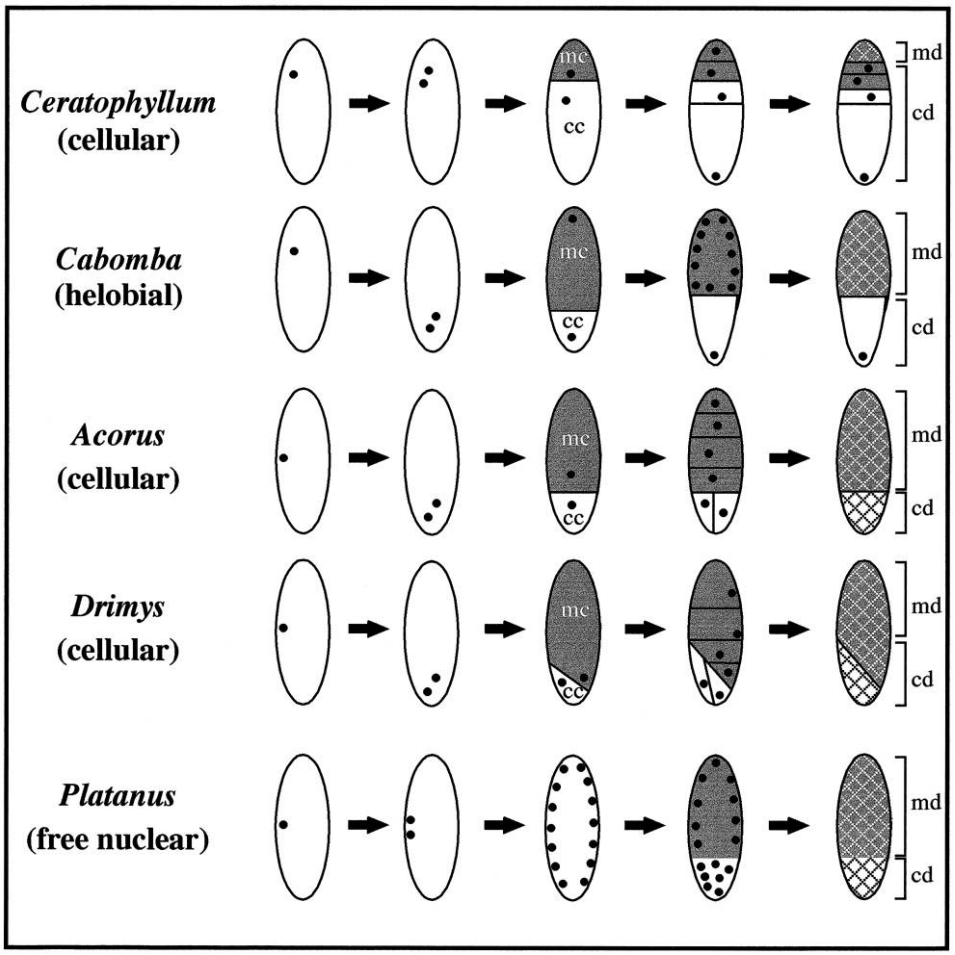


**Fig. 18** Diagrammatic comparison of endosperm development in *Ceratophyllum*, *Cabomba*, *Acorus*, *Drimys*, and *Platanus* as described in the text. Grey shading indicates the part of the endosperm derived from the micropylar cell. Unequal partitioning of the first endosperm cell, differential development of chalazal and micropylar domains, and differential contribution of the two regions to the mature endosperm occur in all taxa. In *Ceratophyllum* and *Drimys*, the micropylar domain (*md*) is derived exclusively from derivatives of the micropylar cell (*mc*), and the chalazal domain (*cd*) is derived from both the chalazal (*cc*) and micropylar cells. In both *Cabomba* and *Acorus*, the micropylar domain is derived entirely from the micropylar cell, and the chalazal domain is derived from the chalazal cell. In *Platanus*, there is no initial cellular partition.

differential development in the early patterning of female gametophytes in nonflowering seed plants, except for the highly apomorphic *Gnetum* (Friedman and Carmichael 1998). In contrast, the embryo-like developmental properties of endosperm in angiosperms, both basal and highly nested taxa, are highly intriguing and potentially congruent with an embryo homology for the endosperm (also suggested by Kranz 1998). An in-depth comparative analysis of the ontogeny of primitive endosperm, seed plant embryos, and female gametophytes may indeed provide new clues about the ancestral homologue of endosperm.

## *Conclusions*

The origin and early evolution of the flowering plants is intimately associated with the origin and evolution of the features that allow us to so easily distinguish them from other seed plants. That Darwin's "abominable mystery" remains unsolved after more than a century attests to the difficulty of tracing the evolutionary history of defining angiosperm characters. By taking an explicitly phylogenetic approach to the selection of taxa and analysis of character distribution, as well as abandoning the limits of typological categorization of development, it has been possible to construct an explicit hypothesis for the primitive bipolar cellular nature of endosperm and a framework for understanding the ontogenetic evolution of endosperm. We have also been able to discern patterns of character evolution for endosperm that occurred during early angiosperm diversification. By establishing a plesiomorphic endosperm ontogeny and the basis for reconstructing the evolutionary transformation of that developmental pattern, this work provides new insight into the origin and evolution of a significant novel feature that is associated with one of the most important events in the generation of modern biodiversity, the origin of angiosperms. We hope that this analysis of endosperm

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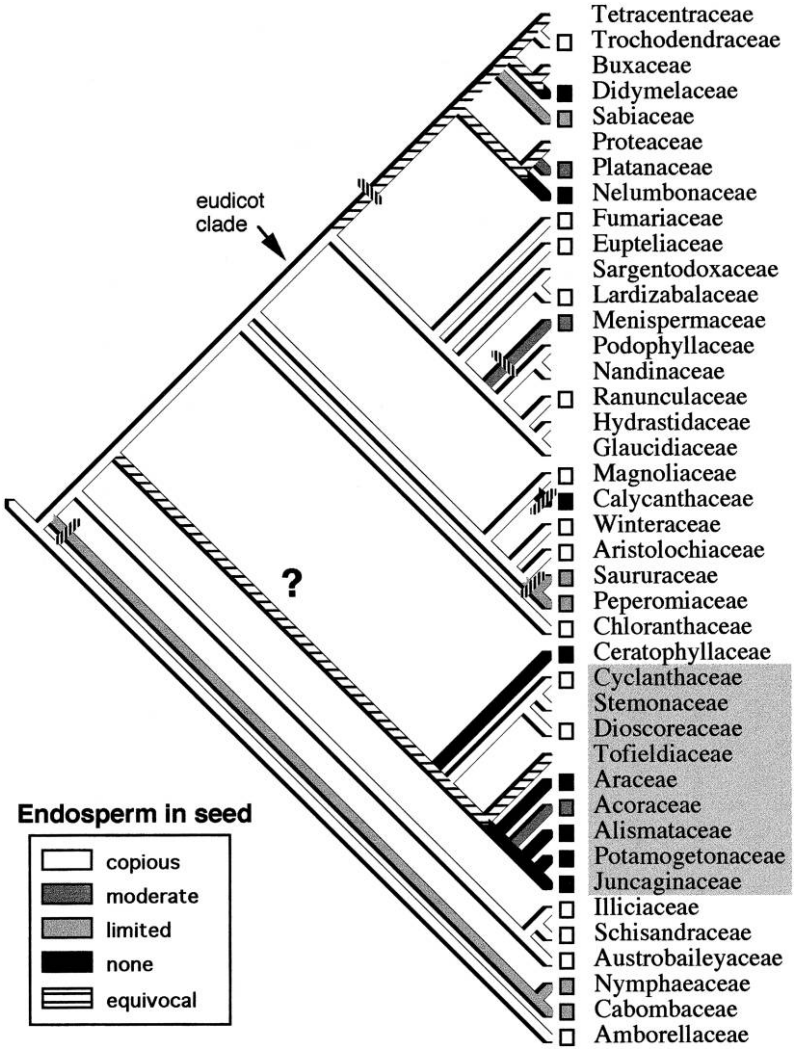


**Fig. 19** Endosperm in the mature seed parsimoniously optimized onto the phylogeny of Qiu et al. (1999), with the origin of nonendosperm storage tissue mapped on as tick marks. Shaded box indicates the monocot clade. Data for taxa not included in this study are from Cook (1902, 1906, 1909), Mabberley (1987), Grayum (1991), and Johri et al. (1992). Nonendosperm storage tissues (either embryo or nucellar) have evolved independently in the same lineages in which endosperm has been reduced from a plesiomorphic "copious" state. Exact point of transition of both characters is equivocal in the monocots.

**Table 5**

**Six Basic Endosperm Developmental Patterns Resulting from Six Combinations of Character States for the Three Components of Early Pattern Formation and Examples of Taxa in Which These Combinations Occur**

| Developmental pattern | PEN division | Micropylar development | Chalazal development | Type | Examples |
|---|---|---|---|---|---|
| 1 ..... | Cellular | Cellular | Cellular | *Ab initio* cellular | *Acorus*, *Drimys*, Araceae |
| 2 ..... | Cellular | Free nuclear | Free nuclear | Helobial | Araceae, Alismataceae |
| 3 ..... | Cellular | Free nuclear | Cellular | Helobial | Araceae |
| 4 ..... | Free nuclear | Free nuclear | Free nuclear | Free nuclear | Lauraceae, *Platanus*, Araceae |
| 5 ..... | Cellular | Free nuclear | None | Helobial | *Cabomba*, Araceae |
| 6 ..... | Cellular | Cellular | None | *Ab initio* cellular | *Saururus*, Nymphaeaceae, Araceae |

Note. Data for taxa not included in this analysis from Grayum (1991), Johri et al. (1992), and Heo et al. (1998).

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in basal angiosperms takes us one step closer to revealing the enigmatic early history of the flowering plants.

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