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Embryology of *Amborella* (Amborellaceae): Descriptions and Polarity of Character States

Hiroshi Tobe[1]*, Tanguy Jaffré[2] and Peter H. Raven[3]

[1] *Department of Botany, Graduate School of Science, Kyoto University, Kyoto, 606-8502 Japan*
[2] *Botany Department, IRD, BP A5, Nouméa, New Caledonia*
[3] *Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166, U.S.A.*

We present the first study on the embryology of *Amborella trichopoda* Baill. which, based on recent analyses of multiple genes, was identified as the first branch in the angiosperm evolution. Comparisons with two other basal angiosperms (Nymphaeaceae and Illiciales), as well as with the other angiosperms, showed that most of the embryological features of *Amborella* are plesiomorphies. They include: the tetrasporangiate anther; the fibrous endothecium; the glandular tapetum composed of two-nucleate cells; two-celled mature pollen; formation of the hypostase; bitegmic and crassinucellate ovules; the Polygonum type embryo sac; ephemeral antipodal cells; a lack of vascular bundles in the integuments; *ab intio* Cellular type endosperm; non-pachychalzal ovule or seed; albuminous mature seed. Contrary to all previous descriptions, *Amborella* has hemianatropous, rather than anatropous or orthotropous, ovules. For the determination of the polarity of ovule curvature as well as of some other characters such as the presence or absence of the nucellar cap and the obturator, the formation of the micropyle, and seed coat structure, additional studies on Nymphaeaceae and Illiciales are needed.

Key words: ***Amborella*** **— Amborellaceae — Embryology**

The genus *Amborella* comprises a single species *A. trichopoda* Baill., which occurs as dioecious shrubs in the undergrowth of rain forests at relatively high elevations (200-1,000 m) in New Caledonia (Jérémie 1982). Until half a century ago, based on morphological similarities of male flowers, the genus had been placed in Monimiaceae (Baillon 1873, Bentham and Hooker 1883, Pax 1889, Perkins 1898, 1925, Perkins and Gilg 1901). Thereafter, because of the lack of vessels (Bailey and Swamy 1948, Bailey 1957), the genus has been treated as a distinct family Amborellaceae but considered closely related to Monimiaceae (Buchheim 1964, Cronquist 1981, Dahlgren 1989, Thorne 1992, Takhtajan 1997). Contrary to these traditional taxonomic treatments, most recent molecular phylogenetic analyses based on sequences of mitochondorial, plastid, and/or nuclear DNA have revealed that *Amborella* represents the first branch of the angiosperm evolution and thus is sister to all the other angiosperms (Mathews and Donophue 1999, Soltis *et al.*1999, Qiu *et al.* 1999, Parkinson *et al.* 1999). These results have attracted attention to the morphological characters of *Amborella* which might represent plesiomorphies in the angiosperm evolution.

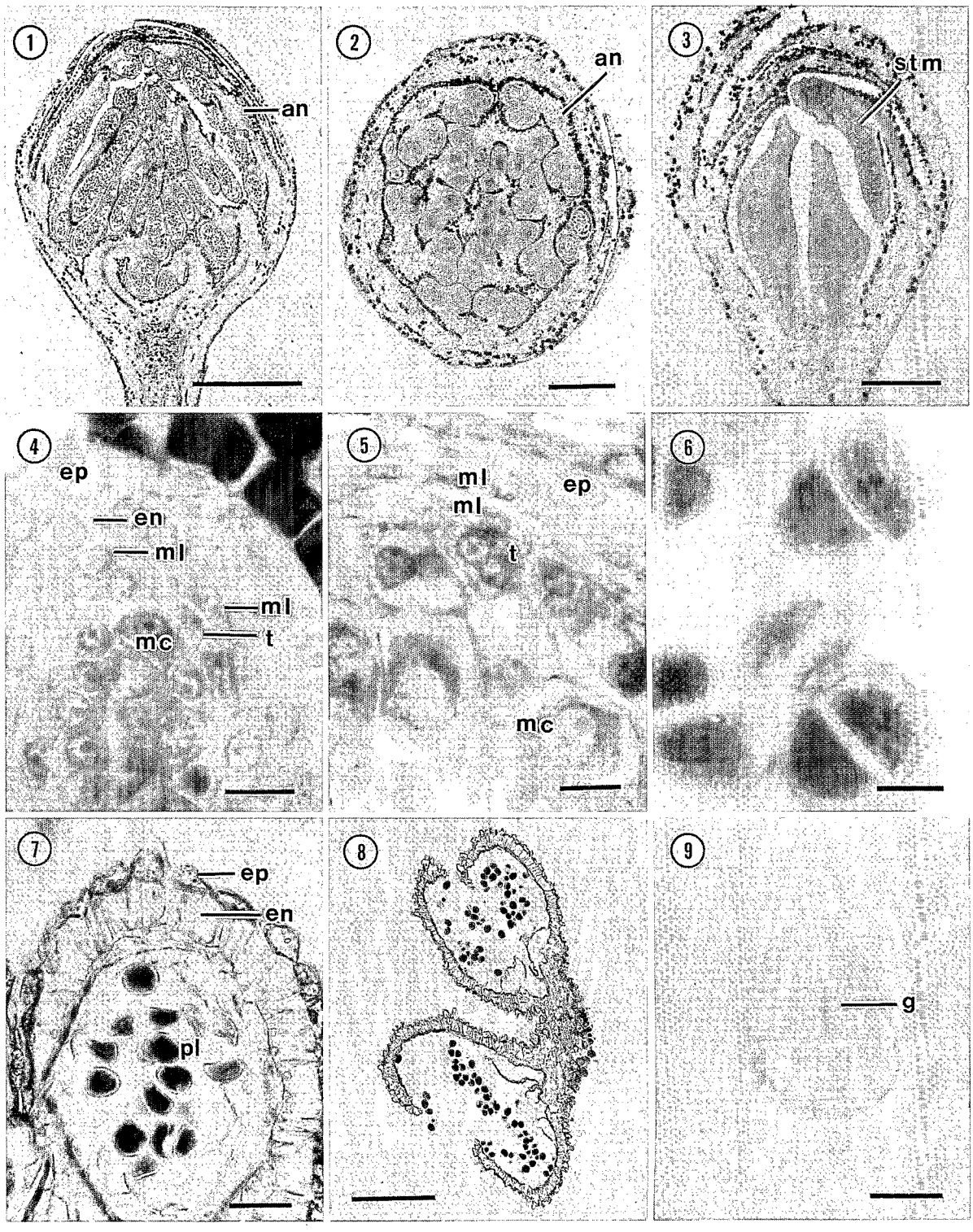
The purpose of this paper is to document developmental features of embryological characters in *Amborella* which have been poorly understood to date. A few characters of ovule and seed structure have been described by other workers (e.g., Perkins and Gilg 1901, Bailey and Swamy 1948, Endress 1994, Endress and Igersheim 1997). However, since they were not based on observations of the development, several characters like ovule curvature are not well understood. Many other embryological characters are yet to be described. We also compare embryological features of *Amborella* with those of Nymphaeaceae and Austrobaileyaceae (Illiciales), two other basal angiosperms (Mathews and Donophue 1999, Soltis *et al.* 1999, Qiu *et al.* 1999, Parkinson *et al.* 1999), as well as with those of the other angiosperms, and discuss the polarity of each character state. To know plesiomorphies and apomorphies of the embryological characters in the most basal angiosperms will provide us a sound basis for understanding the angiosperm evolution.

Materials and Methods

Our primary observations of *Amborella trichopoda* were based on female buds and flowers collected from trees cultivated at the Arboretum of the University of California, Santa Cruz, U.S.A. in 1993 (no voucher) and on mature fruits collected at the Aoupinié mountain in 1993 (no voucher). In addition, male and female flower buds and fruits were collected in the fields at Massif de Dogny near Sarramea, Povila forest near Poindimié, and Mont Aoupinié near Ponérihouen in New Caledonia in 1999 and 2000 (vouchers: *AZ00031001, AZ0031002, AZ00031101, AZ00031301, NC134, NC135, NC159, NC160*, KYO). The flower buds, open flowers and fruits were all fixed with FAA (five parts stock formalin; five parts glacial acetic acid; 90 parts 50% ethanol).

For the present study, 15 male flower buds and open

*Corresponding author



Figs. 1–9. Development of anther and microspores in *Amborella*. 1. Longitudinal section (LS) of male flower. 2. Transverse section (TS) of male flower. 3. LS of female flower with a staminodium. 4. TS of anther. 5. TS of microsporangium showing two-nucleate glandular tapetal cells. 6. Meiosis in microsporangium showing successive cytokinesis. 7. TS. of older anther showing fibrous thickenings of the endothecium. 8. Microtome section of dehiscing anther. 9. Mature pollen stained by acetocarmine. Abbreviations: an, anther; end, endothecium; ep, epidermis; g, nucleus of a generative cell; mc, microspore mother cell; ml, middle layer; pl, pollen; stm, staminodium; t tapetum. Scale bars equal 1 mm in Fig. 1, 300 μm in Figs. 2 and 3, 30 μm in Fig. 7, 20 μm in Fig. 8, and 10 μm in Figs. 4–6 and 9.

flowers, 87 female flower buds and open flowers, and 32 seeds or fruits in various developmental stages were dehydrated through a *t*-butyl alcohol series, embedded in Paraplast (melting point, 57–58 C), and sectioned using a rotary microtome following standard paraffin methods. Sections cut at about 6–10 m thick were stained with Heidenhain's haematoxylin, Safranin-O and FastGreen FCF, and mounted with Entellan. To count the number of cells in mature pollen grains, pollen was stained with 1% acetocarmine (Tobe and Raven 1984).

Results

Anthers and microspores

In male flowers 13 to 19 stamens are borne from the inner surface of the receptacle (Figs. 1, 2). The tetrasporangiate anther is supported by a relatively short filament (Fig. 2). Female flowers have one or two staminodia which are morphologically similar to the stamens in male flowers (Fig. 3), and although many sporogenous cells appear to differentiate in the anther of the staminodium, they show no sign of meiotic divisions. In the male flowers, the anther wall comprises five to six cell-layers prior to maturation: an epidermis, an endothecium, two to three middle layers, and a tapetum (Fig. 4). The middle layers share their histogenetic origin with both the endothecial and the tapetal cells (i.e., Basic type). The tapetum is glandular, and its cells are two-nucleate (Fig. 5). During maturation, the epidermal cells are enlarged to some extent and accumulate tannin-like substances, and the endothecium develops fibrous thickenings (Figs. 7, 8). The middle layers degenerate (Fig. 5). Anther dehiscence takes place by longitudinal slits, with each slit common to two microsporangia of a theca (Fig. 8).

Meiosis in the microspore mother cell is accompanied by successive cytokinesis (Fig. 6). The shape of resultant tetrads is tetrahedral, decussate, or isobilateral. Pollen grains are two-celled at the time of shedding as shown in Fig. 9, where only a nucleus of the generative cell is stained.

Ovules and integuments

The female flower buds and open flowers examined have four or five separate carpels (Figs. 10, 11). Each carpel has a single ovule; no cases existed where two or more ovules are borne in the carpel.

The ovule is hemianatropous, and is neither anatropous as described by Bailey and Swamy (1948) nor "almost orthotropous" as described by Endress (1994), and Endress and Igersheim (1997). The ovule primordium is initiated from the lateral side of the carpellary locule and the apex is oriented toward a horizontal line (Fig. 12). As the ovule develops, it curves downward (Figs. 13–15) and eventually the nucellus forms an angle of 90 degrees with the funicle (Fig. 16). Thus the axis of the ovule is not straight, and in this respect the ovule of *Amborella* is different from the true orthotropous ovule (with a straight axis) as in Juglandaceae (e.g. Johri *et al*. 1992, p. 118).

The ovule is bitegmic, having an inner and an outer integument (Figs. 14–19). Both the inner and the outer integuments are initiated by periclinal divisions of dermal cells of the ovlue primordium. The inner integument is two cells thick throughout except at the micropylar part where it is four to five cells thick as described below and at the basal part where it is three cells thick (Fig. 17). On the other hand, the outer integument is four to seven cells thick on the funicular (or dorsal) side, while it is two to three cells thick on the anti-funicular (or ventral) side (Fig. 18). Therefore the outer integument is thicker on the funicular side than on the anti-funicular side as is common in anatropous ovules.

No endothelium is formed.

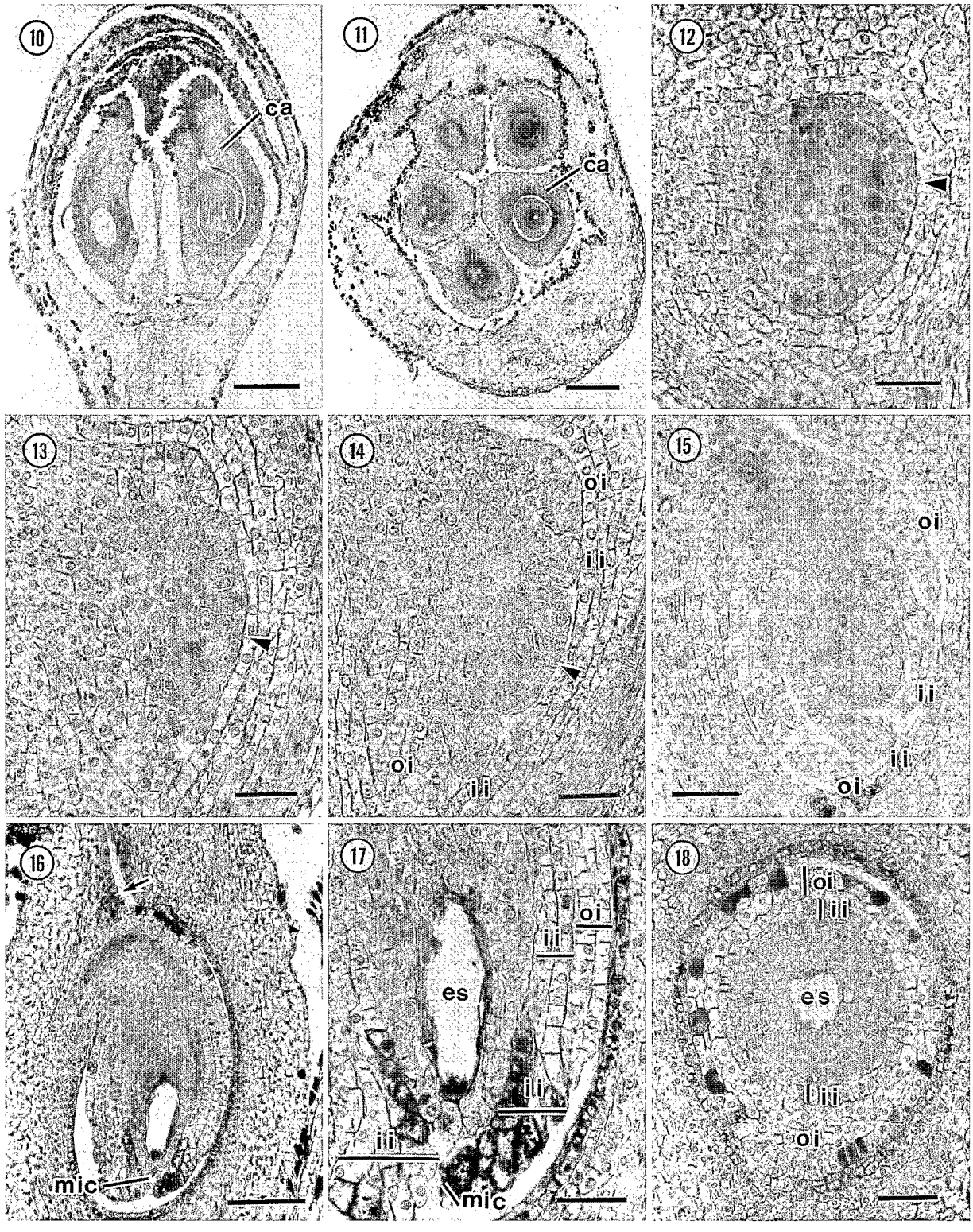
A single vascular bundle supplies the ovule, and it ends at the chalazal region and ramifies there as the seed develops (see Fig. 32). Neither integument has vascular bundles. The ovule or seed is not pachychalazal.

The micropyle is formed by the inner integument alone (Figs. 16, 17). When the inner integument forms the micropyle, cells of its tip divide anticlinally so that the tip of the inner integument becomes four to five cells thick (Fig. 17). At the micropyle, cells of the inner epidermis and adjacent ones of the inner integument are usually tanniniferous (Fig. 17), forming a cap-like, permanent structure (see Figs. 34, 36). No aril (caruncle) is formed from the outer integument. No obturator-like structure develops on the tip of the outer integument on the funicular side.

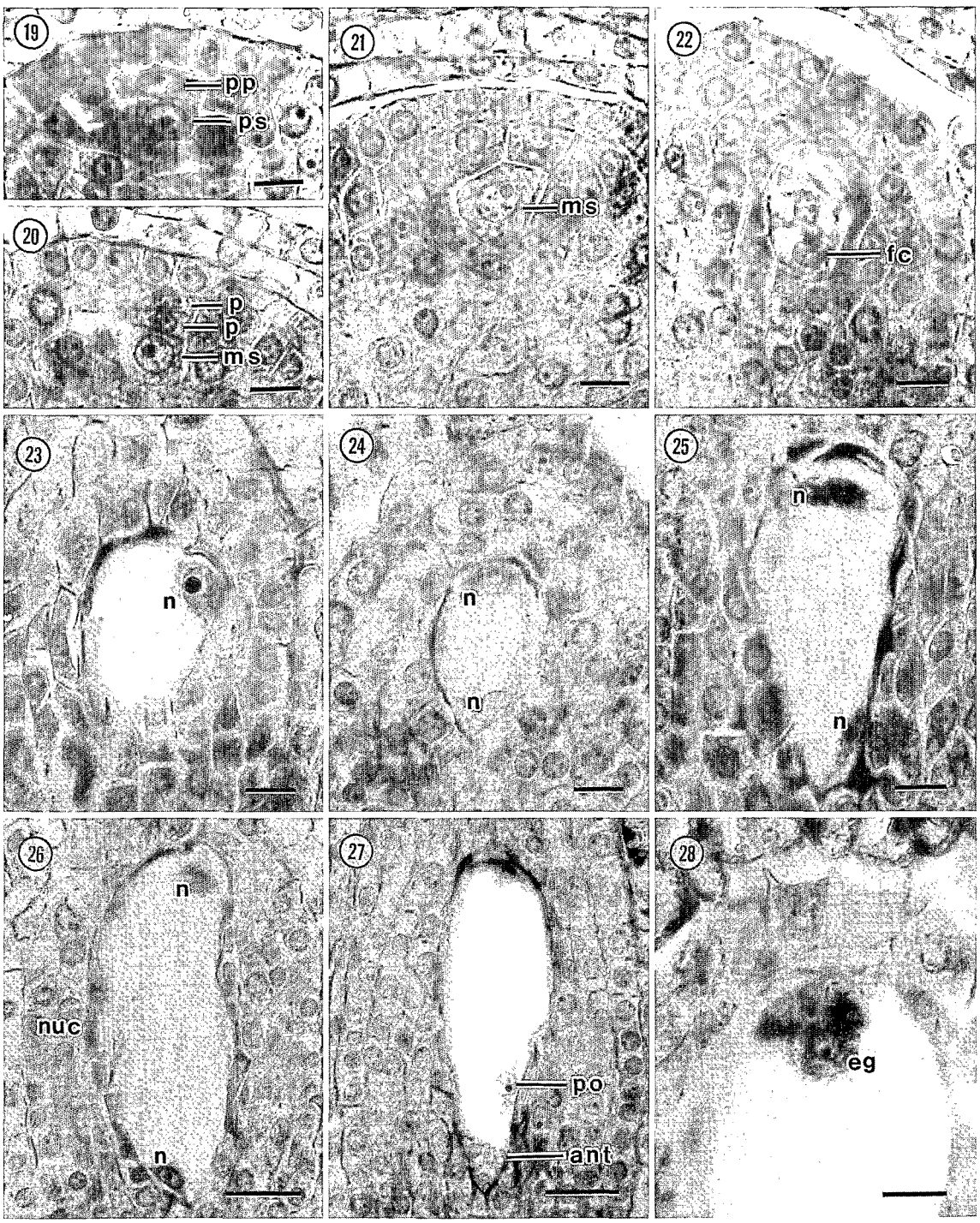
Nucellus and megagametophyte

The nucellus is crassinucellate. A single archesporial cell is differentiated at the hypodermis of the nucellar apex, and it divides periclinally into the primary parietal cell and the primary sporogenous cell (Fig. 19). The primary parietal cell further divides into two parietal cells (Fig. 20), while the primary sporogenous cell differentiates into the megaspore mother cell and increases its volume (Fig. 21). The megaspore mother cell undergoes meiosis to form a linear or T-shaped tetrad of megaspores (Fig. 22). While the three upper megaspores degenerate, the chalazal megaspore functions to form an one-nucleate embryo sac first (Fig. 23) and, by nuclear divisions, develops successively into a two-nucleate (Figs. 24, 25), a four-nucleate (Fig. 26), and an eight-nucleate embryo sac. Thus the embryo sac formation conforms to the Polygonum type. Throughout the development of the embryo sac, nuclei are positioned with little cytoplasm at the extreme periphery in the enlarging embryo sac. An organized embryo sac is composed of an egg cell, two synergids, two polar nuclei, and three antipodal cells (Figs. 27, 28). The two polar nuclei are usually positioned in the chalazal region and fuse to form a single central nucleus before fertilization. In microtome sections of many mature ovules, the synergids always appeared less conspicuous (Fig. 28). The three antipodal cells are cellularized at the chalazal end but degenerate probably before fertilization because most of the mature embryo sacs lacked them.

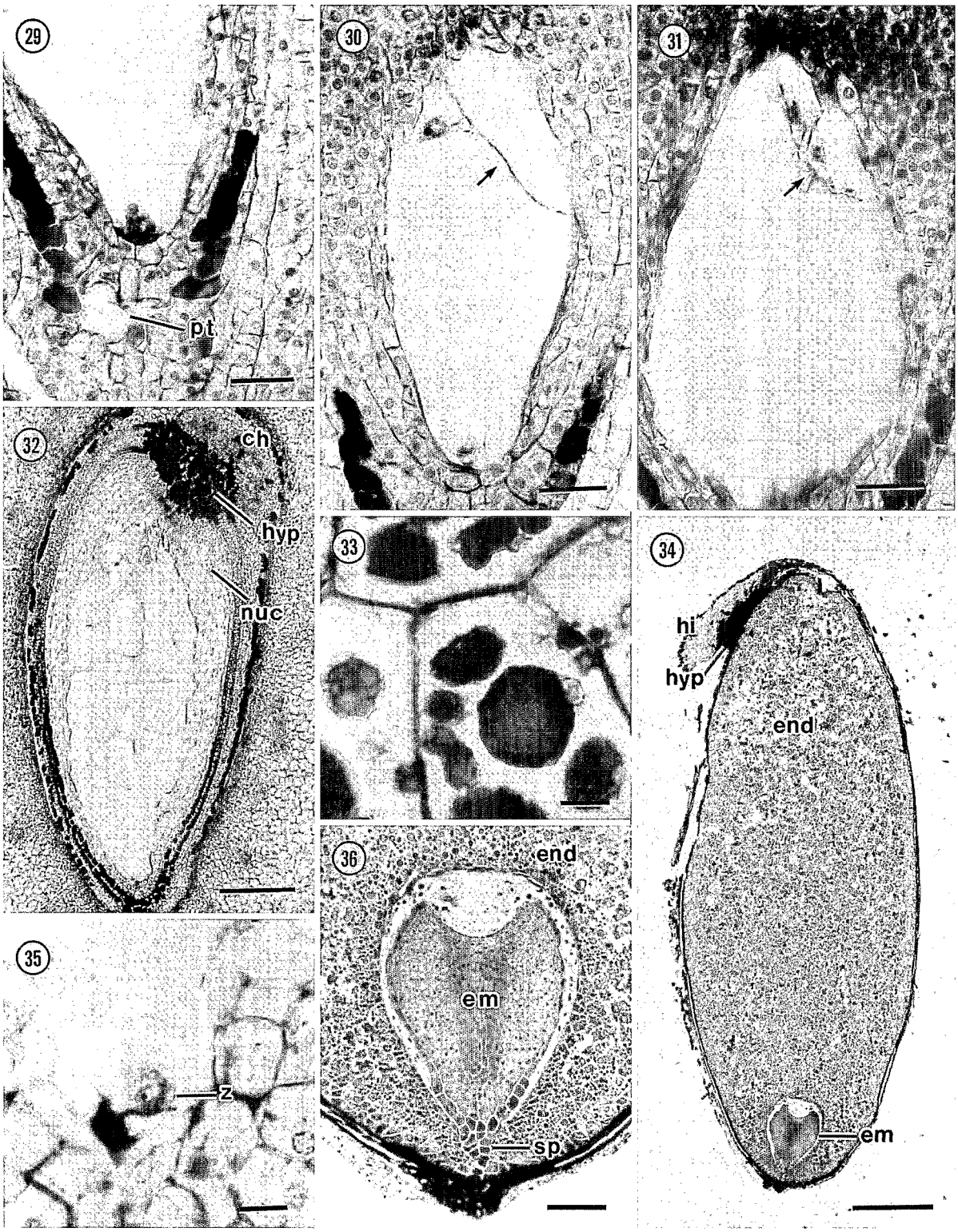
Apical epidermal cells of the nucellus do not divide periclinally. The nucellar tissue surrounding the mature embryo sac remains until post-fertilization stages (Figs. 27, 32).



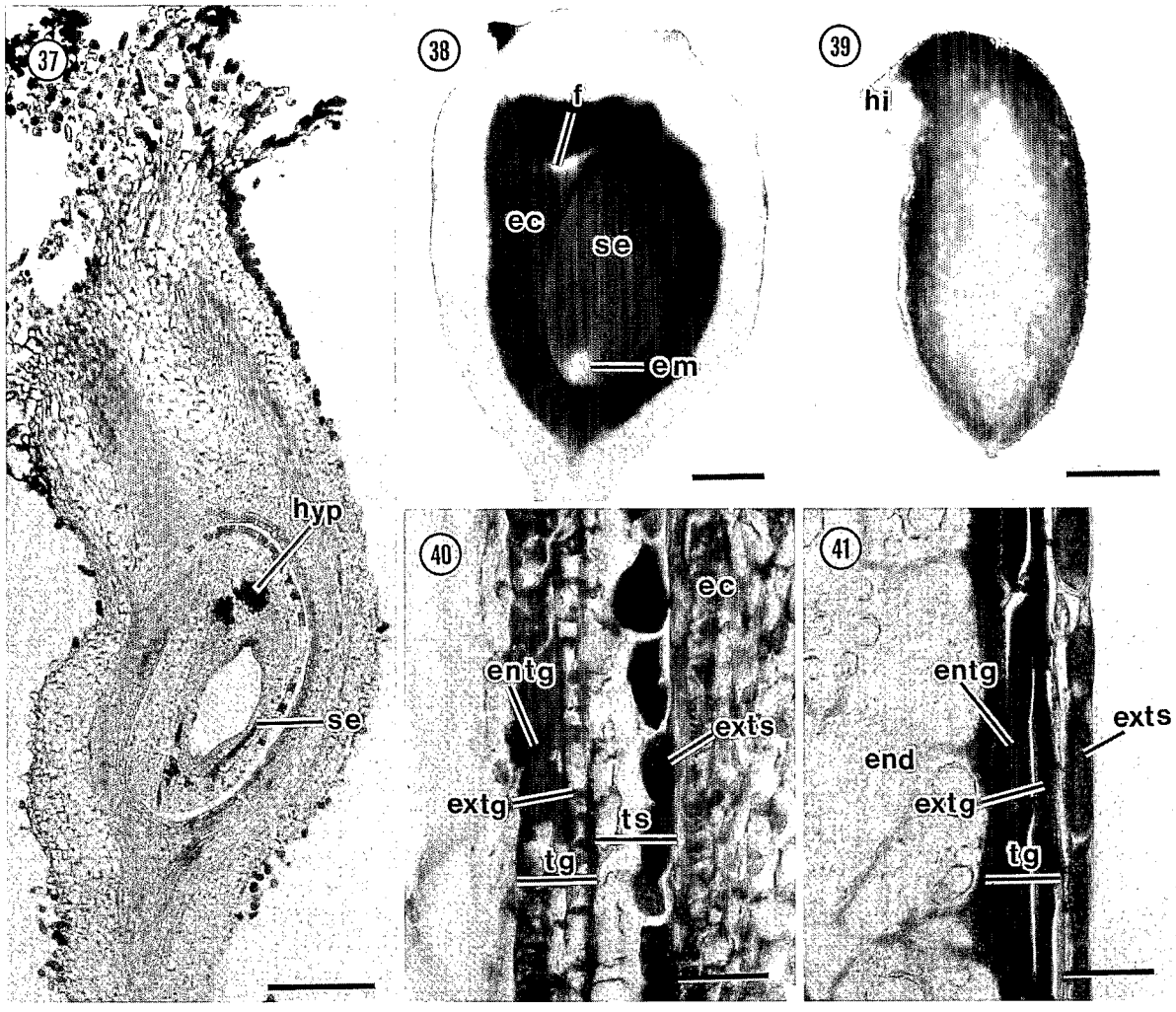
Figs. 10-18. Development of ovule and integuments in *Amborella*. 10. Longitudinal section (LS) of female flower. 11. Transverse section (TS) of female flower. 12-16. LSs of young to mature ovules. In all photos, flowers (flower buds) are presented exactly vertically to show gradual changes of the position of a nucellar apex (arrowhead). An arrow in Fig. 16 indicates a transmitting tissue densely stained. 17. LS of mature ovule showing a micropyle formed by the iner integument alone. 18. TS of mature ovule. The anti-funicular side is presented at the top, and the funicular side at the bottom. Abbreviations: ca, carpel; es, embryo sac; ii, inner integument; mic, micropyle; oi, outer integument. Scale bars equal 300 μm in Figs. 10 and 11, 50 μm in Figs. 15 and 18, and 30 μm in Figs. 12-14 and 17.



Figs. 19-28. Development of nucellus and embryo sac in *Amborella*. 19. Longitudinal section (LS) of young ovule, in which an archesporial cell is periclinally divided (see Fig. 12). 20. LS of young ovule with a young megaspore mother cell (see Fig. 13). 21. LS of ovule with a megaspore mother cell (see Fig. 14). 22. LS of ovule with a tetrad of megaspores, in which the chalazal megaspore is functional. 23. LS of older ovule with an one-nucleate embryo sac. 24. LS of older ovule with a two-nucleate embryo sac. 25. LS of older ovule showing that nuclei in the two-nucleate embryo sac are dividing. 26. LS of older ovule with a four-nucleate embryo sac showing only two nuclei in the photo. 27. LS of mature ovule with an organized embryo sac showing only three antipodal cells and one polar nucleus in the photo. 28. LS of mature ovule showing the egg apparatus. Abbreviations: ant, antipodal cell; eg, egg cell; fc, functioning megaspore; ms, megaspore mother cell; n, nucleus of embryo sac; nuc, nucellar tissue; p, parietal cell; po, polar nucleus; pp, primary parietal cell; ps, primary sporogenous cell. Scale bars equal 20 μm in Figs. 26 and 27, and 10 μm in Figs. 19-25 and 28.



Figs. 29–36. Development of endosperm and embryo in *Amborella*. 29. Longitudinal section (LS) of fertilized ovule showing the path of a pollen tube. 30. LS of young seed showing the first division of an endosperm nucleus. An arrow in Figs. 30 and 31 indicates the first cell wall. 31. LS of young seed showing subsequent divisions of endosperm cells. 32. LS of young seed with some endosperm cells. 33. Microtome section of endosperm cells in a mature seed showing protein bodies. 34. LS of mature seed. 35. A zygote found in the same young seed as the one presented in Fig. 32. 36. LS of embryo in the mature seed. Abbreviations: ch, chalaza; em, embryo; end, endosperm; hi, hilum; hyp, hypostase; nuc, nucellar tissue; pt, pollen tube; sp, suspensor; z, zygote.



Figs. 37–41. Development of seed and seed coat in *Amborella*. 37. Longitudinal section (LS) of young fruit. 38. Longitudinal hand-section of a mature fruit. 39. Mature seed. 40. LS of young seed coat. LS of mature seed coat. Abbreviations: ec, endocarp; em, embryo; end, endosperm; entg, endotegmen; extg, exotegmen; exts, exotesta; hi, hilum; se, seed; tg, tegmen; ts, testa. Scale bars equal 1 mm in Figs. 38 and 39, 300 μm in Fig. 37, and 20 μm in Figs. 40 and 41.

The hypostase is formed after fertilization (Figs. 32, 34, 37).

Fertilization, endosperm and embryo

Fertilization is porogamous (Fig. 29). When the ovule reached maturity, some epidermal cells of the carpellary canal just above the level of attachment of the ovule appeared to contain much cytoplasm because they stained densely with haemetoxylin (Fig. 16, an arrow). These cells probably function as the transmitting tissue to conduct a pollen tube toward the ovule.

Endosperm formation is of *ab initio* Cellular type. The first division of the primary endosperm nucleus occurs near the chalazal end and the cell wall forms obliquely (Fig. 30). Subsequent divisions also occur on the chalazal side (Figs. 31, 32) but eventually in mature seeds the embryo sac is entirely occupied by endosperm cells (Fig. 34). The endosperm cells accumulate abundant lipids and protein (Fig. 33).

The embryogenesis proceeds very slowly. Even in a seed with hundreds of endosperm cells, a zygote still remains undivided (Fig. 35). In older and mature seeds, an embryo is small and enclosed by copious endosperm (Fig. 36). The embryo is dicotyledonous with a short suspensor (Fig. 36).

Seed and seed coat

The seed is pendulous and develops within the indehiscent fruit wall (Figs. 37, 38). Mature seeds are ellipsoid in shape, and 3.8–4.2 mm long and 1.6–2.0 mm in diameter (Figs. 38, 39), and their surface is dark-brown due to the color of the exotesta.

While the fruit wall develops a thick endocarp (Fig. 38), the seed coat develops as a thin layer. The young seed coat is formed of a two to three cell-layered tegmen and a two to three cell-layered testa. In the tegmen, at first, only the inner epidermis (endotegmen) becomes tanniniferous.

Table 1. Comparisons of *Amborella* in embryological characters with Nymphaeaceae, Austrobaileyaceae, and other angiosperms

Characters	*Amborella*	Nymphaeaceae	Austrobaileyaceae	Other angiosperms
Anthers and microspores				
Number of sporangia	4	4	4	2, 4, or more
Thickness of anther wall	5-6 cell-layered	5-8 cell-layered	5-8 cell-layered	Various
Mode of wall formation	Basic	NA	NA	Basic, Monocotyledonous, Dicotyledonous, Reduced
Anther epidermis	Persistent	NA	Persistent	Persistent or collapsed
Endothecium	Fibrous	Usually fibrous or rarely non-fibrous	Fibrous	Fibrous or non-fibrous
Middle layers	Crushed	Crushed or persistent	Persistent	Crushed or persistent
Tapetum	Glandular	Glandular or amoeboid	Glandular	Glandular or amoeboid
Number of nuclei in tapetal cell	2	2 or more	1 or 2	1, 2, or more
Cytokinesis in meiosis	Successive	Successive or simultaneous	Simultaneous	Successive or simultaneous
Shape of microspore tetrads	Tetrahedral, decussate, isobilateral	Tetrahedral or isobilateral	NA	Tetrahedral, etc.
Mature pollen	2-celled	2- or 3-celled	2-celled	2- or 3-celled
Ovules and integuments				
Ovule orientation	Hemianatropous	Anatropous, or rarely orthotropous	Anatropous	Anatropous, campylotropous, hemianatropous, orthotropous
Number of integuments	2	2	2	1 or 2
Thickness of ii (early stage)*	2 cell-layered	2 cell-layered	2(-3) cell-layered	2 to many cell-layered
Thickness of ii (late stage)*	2 cell-layered	2 cell-layered	2(-3) cell-layered	2 to many cell-layered
Thickness of oi (early stage)*	2 cell-layered	NA	NA	2 to many cell-layered
Thickness of oi (late stage)*	2 cell-layered	2-6 cell-layered	6 or more cell-layered	2 to many cell-layered
Vascular bundles	Absent	Absent	Present at the base of oi	Absent, or present in ii or oi
Micropyle formation	By ii	By oi	By ii, or both ii and oi	By ii, oi, or both ii and oi
Endothelium	Not formed	Not formed	Not formed	Not formed or formed
Obturator	Not formed	NA	NA	Not formed or formed
Nucellus and megagametophyte				
Nature of nucellus	Crassinucellate	Crassinucellate	Crassinucellate	Crassinucellate or tenuinucellate
Number of archesporial cells	1	1	1	1 to many
Thickness of parietal tissue	2 cells thick	1 to 6 cells thick	3-4 cells thick	0 to many cells thick
Mode of embryo sac formation	Polygonum	Polygonum	Polygonum	Polygonum, Allium, etc.
Antipodal cells	Ephemeral	Ephemeral	NA	Ephemeral or persistent
Nucellar cap	Not formed	NA	Formed	Not formed or formed
Nucellar tissue in mature ovule	Present	Present	Present	Present or absent
Hypostase	Formed	Formed	NA	Formed or not formed
Endosperm and embryo				
Mode of endosperm formation	*ab initio* Cellular	*ab initio* Cellular or Nuclear	NA	*ab initio* Cellular, Helobial, Nuclear
Type of embryogeny	NA	NA	NA	Asterad, Onagrad, etc.
Seed and seed coat				
Pachychalazy	No	No	No (perichalazal)	No or yes
Arl/wing	Not formed	Rarely formed (aril)	Not formed	Formed or not formed
Endosperm in mature seed	Copious	Present (small amount) to absent	Copious	Copious to absent
Type of seed coat	Exotestal-endotegmic	Mostly exotestal	NA	Various
Thickness of testa	1 cell thick	Usually 1 cell thick	NA	Various
Cells of exotesta	Thick-walled, Lignified, flattened	Thick-walled, lignified	NA	Various
Cells of endotesta	Crushed	Crushed	NA	Various
Thickness of tegmen	2 cells thick	NA	NA	Various
Cells of exotegmen	Thick-walled, lignified, collapsed	Crushed	Crushed ?	Various
Cells of endotegmen	Thick-walled, lignified, flattened	Crushed	Crushed ?	Various
References	Present study, Endress and Igersheim (1997)	Padmanabhan (1970), Corner (1976), Johri *et al.* (1992, pp. 295-300)	Endress (1980)	Corner (1976), Johri *et al.* (1992)

*The thickness is measured on the anti-funicular (or dorsal) side.

Abbreviations: ii, inner integument; oi, outer integument; Na, data not applicable.

Later, however, the outer epidermis also becomes tanniniferous (Fig. 40). In the testa, the outer epidermis (exotesta) accumulates tannin-like substances which look dark-brown (Fig. 40). As the seed matures, cells of the inner epidermis of the testa are crushed. The mature seed coat thus comprises three cell-layers: the endotegmen, exotegmen, and exotesta (Fig. 41). Cells of the endotegmen and exotesta are thick-walled, lignified and rather enlarged longitudinally, while cells of the exotegmen are also lignified but collapsed. Thus the seed coat of *Amborella* is endotegmic-exotestal (for terminology see Schmid 1986).

Discussion

All the embryological characters described for *Amborella* have two or more character states and thus have been used as evidence for relationships at various taxonomic levels, from the ordinal to the specific (for review see Tobe 1989). In order to determine the polarity of their respective character states, a few embryological characters have been examined, such as the number of cells in mature pollen (two-celled vs. three-celled), the number of integuments (one vs. two), tapetum type (glandular vs. amoeboid), and the endosperm formation mode (Nuclear vs. Cellular) (e.g., Brewbaker 1967, Dahlgren 1991). Previous attempts, however, were based on mapping the character states in a morphology-based phylogenetic tree or diagram of angiosperms. This method must be supported by a robust phylogenetic tree or diagram as well as the extensive availability of embryological data. For the last decade, considerable changes have been proposed based on molecular phylogenetic analyses to the traditional, morphology-based system of classification (e.g., Angiosperm Phylogeny Group 1998); in addition, embryological data are still lacking or insufficient for more than 30% of angiosperm families (Tobe 1989).

In the phylogenetic trees generated by molecular analyses using mitochondorial, plastid, and/or nuclear DNA sequences (Mathews and Donophue 1999, Soltis *et al.*1999, Qiu *et al.* 1999, Parkinson *et al.* 1999), relationships of basal angiosperms, i.e., *Amborella* (Nymphaeaceae (Illiciales-other angiosperms)), are consistently supported by highest bootstrap probabilities, allowing us alternative insights into the polarity of the embryological characters. Embryological features of *Amborella*, which are common to all or some Nymphaeaceae as well as to all or some Illiciales (Austrobaileyaceae, Illiciaceae, Schisandraceae, and Trimeniaceae) and other angiosperms, are very likely to be plesiomorphies, and distinct features to be apomorphies. In Table 1 embryological features of *Amborella* are summarized and compared with those of Nymphaeaceae, Austrobaileyaceae (positioned most basally in Illiciales) and other angiosperms. Neither Nymphaeaceae nor Austrobaileyaceae have been fully investigated embryologically, and for Austrobaileyaceae, there is a complete lack of data on the development of endosperm, embryo and seed coat. However, available data does provide some indication of which character states are archaic or the most primitive.

It is evident from Table 1 that many embryological features of *Amborella* are common to all or some Nymphaeaceae as well as to Austrobaileyaceae and/or all or somet of the other angiosperms, and thus are plesiomorphies in the angiosperms. They include: the tetrasporangiate anther; a relatively thick (more than five cell-layered) anther wall; the fibrous endothecium; crushed middle layers; glandular tapetum; two-nucleate tapetal cell; successive cytokinesis during meiosis of pollen mother cells; two-celled mature pollen; formation of the hypostase; differentiation of a single archesporial cell in ovule; a crassinucellate ovule; a Polygonum type of embryo sac formation; ephemeral antipodal cells; the presence of nucellar tissue in mature ovule; the formation of two integuments; a thin (two cell-layered) inner and outer integument; a lack of vascular bundles in integuments; no formation of the endothelium; *ab intio* Cellular type of endosperm formation; non-pachychalzal ovule or seed; no formation of the aril; albuminous mature seed.

We did not tabulate embryological data of the other families of Illiciales (i.e., Trimeniaceae, Schisandraceae, and Illiciaceae). Although none of the three families have been fully studied embryologically, the data available for them indicate that the embryological features enumerated as plesiomorphies are further common to all three, two, or at least one of the families (for embryological data of the three families see Johri *et al.* 1993).

The following are apomorphies if present in the (early) angiosperms: bi- or polysporangiate anther; a thin (three cell-layered or Reduced type) anther wall; non-fibrous endothecium; persistent middle layer(s); amoeboid tapetum; one- or multi-nucleate tapetal cells; simultaneous cytokinesis during meiosis of the pollen mother cell; three-celled mature pollen; a lack of hypostase; differentiation of multiple archesporial cells in the ovule; tenuinucellate ovule; embryo sac formation other than the Polygonum type (e.g., Allium type); persistent antipodal cells; a lack of nucellar tissue in mature ovules; the development of a single integument; a thick (more than three cell-layered) inner and outer integument; the presence of vascular bundles in the integuments; the formation of the endothelium; Nuclear type of endosperm formation; pachychalzal ovule or seed; formation of the aril; exalbuminous mature seed.

Returning to the embryological features of *Amborella*, the hemianatropous ovule is probably an autapomorphy. The polarity of a few remaining characters such as the formation of the nucellar cap, the obturator, the micropyle, the thickness of the parietal tissue, and the seed coat type is not determined at the present stage. To determine the polarity of these characters, we need more extensive data from Nymphaeaceae; likewise, embryological studies on the development of the endosperm, embryo, and seed coat in Austrobaileyaceae as well as intensive studies of Trimeniaceae, Schisandraceae, and Illiciaceae.

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