

INTEGUMENTARY STUDIES IN THE POLYCARPICAE IV LIRIODENDRON TULIPIFERA L.

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SUMMARY

Despite the great differences in type of seed dispersal and seed structure, *Liriodendron* and *Magnolia* show similarity in their ovule ontogeny and in the early phases of seed development. The ovule primordium is 3-zonate; the inner integument is dermal, and the outer integument subdermal in origin. The cells of the inner epidermis of the outer integument may undergo periclinal divisions and differentiate into a sclerotic layer. However, the outer epidermis and the subdermal tissue of the outer integument in *Liriodendron* are not multiplicative and do not differentiate into a fleshy, coloured sarcotesta as in *Magnolia*, but undergo sclerification.

This study is in agreement with DANDY's (1964) subdivision of the family and supports the generally held view that *Liriodendron* is the most advanced member of the Magnoliaceae.

It is posed that the 3-zonate ovule primordium is basic in angiosperms. This clashes with the classical carpel concept, in which the carpel is interpreted as a metamorphosed leaf bearing ovules on its margins or on the adaxial surface of its lamina.

1. INTRODUCTION

With regard to the Polycarpicae it may be stated that more pages of our scientific journals and books are filled with phylogenetic speculations concerning their status as the ancestors of the angiosperms and their interrelationships, than with reliable data concerning their anatomy, morphology, and other characters.

Our knowledge of the seed development and of the structure of the mature seed coat of the Polycarpicae is, likewise, restricted to only a relatively small number of species and is often fragmentary. The older publications have been reviewed by NETOLITZKY (1926). Later our factual knowledge has been broadened, particularly by the studies of Bailey et al., by publications of Indian botanists (see BHANDARI 1971), and recently by CORNER (1976).

It is rather generally accepted (see, e.g., HALLIER 1912, TAKHTAJAN 1959, VAN DER PIJL 1969) that the primitive angiospermous seed is characterised by a very small embryo and copious endosperm, and that in the course of evolution of the angiosperms there are trends towards a reduction or even the complete disappearance of the endosperm in the mature seeds and towards a corresponding increase in size of the embryo.

In his well-known books on the origin and evolution of the angiosperms

TAKHTAJAN (1959, 1969), when discussing the Magnoliales as the most primitive group of the Magnoliophyta, states that the most primitive type of seed coat is found within the Magnoliales. He recognises three different types of testae, viz., (1) the *Magnolia*, (2) the *Liriodendron*, and (3) the *Schisandra-Illicium* type. In this he follows ZAZHURILO (1940), whose studies on seed coat structure were based on the topography of the parenchymatic and sclerotic tissues in the mature seed coat of some magnoliid taxa in relation with the integuments. According to Takhtajan, the most primitive seed coat of the angiosperms is represented by the *Magnolia* type. It is characterised by an outer fleshy sarcotesta derived from the outer epidermal and parenchymatic middle layer of the outer integument (o.i.), and by a stony sclerotesta composed of several layers of isodiametric sclereids developed out of the inner epidermis of the o.i. This type of seed coat is compared to the ones of the primitive Gymnosperms as Cycadales and is supposed to be adapted to an endozoochorous, in particular to an endosaurochorous, mode of dispersal. The seed coat of the *Magnolia* type is also described in detail in Degeneriaceae (SWAMY 1949) and in *Michelia* (PADMANABHAN 1960, MOHANA RAO 1975). The *Liriodendron* type is characterised by two sclerenchymatic layers, an outer one composed of fibre-like, tangentially elongated cells and derived from the outer epidermal and middle layers of the o.i., which is jointed to an inner layer of sclereids developed from the inner epidermis of the o.i. The *Schisandra-Illicium* type of seed coat possesses only an outer sclerified layer of palissade-like, radially elongated, and more or less isodiametric sclereids formed by the outer epidermis and the outermost cells of the middle layer of the o.i. Both the *Liriodendron* and the *Schisandra-Illicium* types are supposed to be derived from the *Magnolia* type.

The embryological publications on *Liriodendron* (MANEVAL 1914, KAEISER & BOYCE 1962) do not give detailed information as regards seed coat development. The present paper claims to present some more details on the initiation and histogenesis of the ovule and integuments and on the seed coat structure in *Liriodendron tulipifera*.

2. MATERIAL AND METHODS

Young flower buds, gynoecia and developing fruits of *Liriodendron tulipifera* L. were collected from the municipal tree nurseries at Frankendael in Amsterdam. Mature fruits were obtained from the botanic garden of Bordeaux. The material was fixed in Craf III, dehydrated in a TBA-series, embedded in paraplast, sectioned at 7 μ m, and stained in a safranin-astra blue combination. On account of their hardness, the mature seeds were embedded in epon, sectioned at 2 μ m and sectioned by hand for specific staining with phloroglucinol, aniline sulphate, Ruthenium Red and Sudan IV.

3. OBSERVATIONS

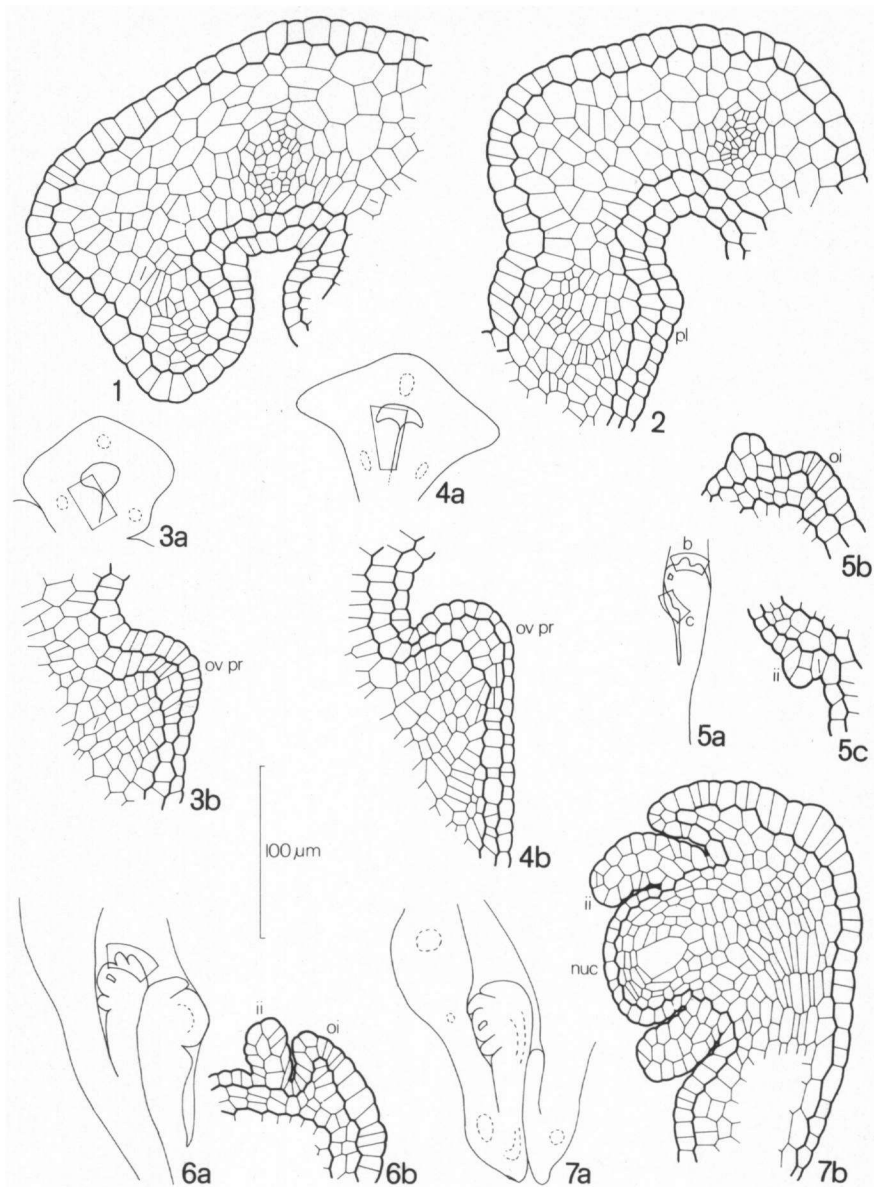
Cross sections of carpel primordia at the placental level (*figs. 1 and 2*) clearly show the three-zonate structure of the adaxial side of the carpel, *i.e.*, two initially only anticlinally dividing layers enveloping a central core. The second zone is not one-layered throughout, some anticlinal divisions occurring especially at the side of the free rim and at the site of the future carpel wing. The adaxial side of the carpel at and above the placenta clearly shows only anticlinal dividing tunica layers before ovule initiation has taken place. The cells of this region have conspicuous nuclei and are rich in cytoplasm. In contrast the cells of the abaxial side of the carpel primordium, including the initials of the carpel wing, are more vacuolised and contain amyloplasts.

Ovule initiation takes place by cell divisions in the central zone of the placenta, which lift the two outer layers (*fig. 3*). However, the second layer also starts dividing periclinally quite soon, especially at the apex of the ovule primordium, the future nucellus (*fig. 4*). These divisions are responsible for the crassinucellate nature of the ovule. Archeporial and megaspore mother cells can only be distinguished by their relative position. During the later developmental stages the single megaspore mother cell becomes discernable by its size and contents. Further periclinal divisions of the primary parietal and neighbouring cells of the second zone give rise to a massive nucellus. The embryo sac becomes covered by a layer of up to twelve parietal cells. Although the nucellus is initially circular in cross section, it becomes more oval during later ontogenetic stages as the result of a higher mitotic activity in the transmedian plane (compare *figs. 11 and 12*). Only the basal tissue of the nucellus, underlying the archesporium, megaspore mother cell, megaspore, or embryo sac, is derived from the corpus. Some of the apical cells of the nucellar epidermis undergo periclinal divisions (*fig. 8*) and form a small nucellar cap which is three to four cells thick.

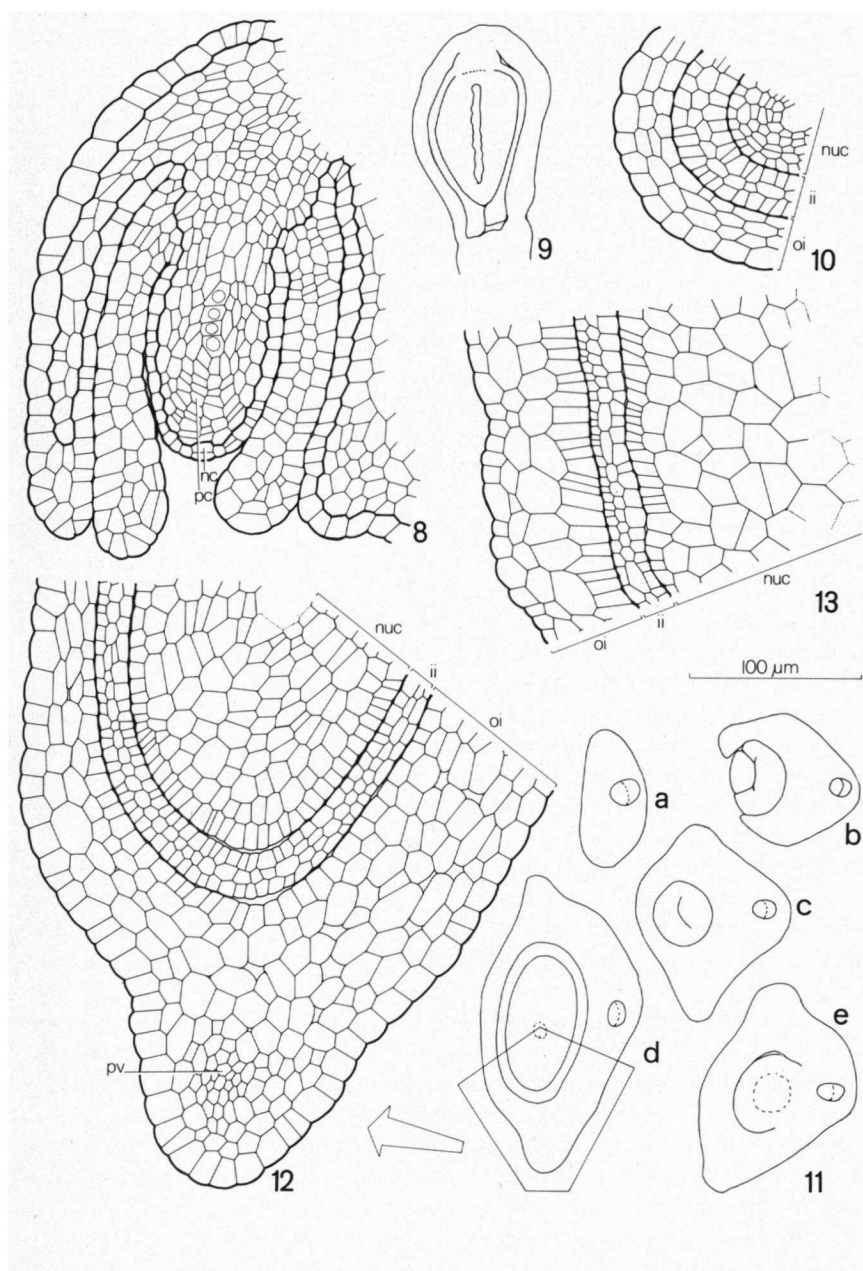
The ovule primordium exhibits a manifest longitudinal growth, caused especially by the mitotic activity of the central zone and giving rise to the well-developed funicle, raphe and chalaza.

It is almost impossible to prepare exactly median, longitudinal sections of ovules showing integument initiation. Although the first growth of the ovule and the starting anatropous curvature is in the median plane of the carpel, the two ovules in each locule facing each other with their backs (the future raphal sides). During further development the ovules become twisted in a plane transmedian in respect of the floral axis so that the mature ovules and seeds are parallel with the floral axis, with their micropyles both oriented upwards.

The inner integument (*i.i.*) is initiated almost simultaneously at all sides of the ovule primordium. Its initiation takes place in the dermal layer (*fig. 5*) as in *Magnolia* and *Drimys*; in a longitudinal section mostly three dermal initials are seen to be involved in its formation. The middle one divides, always in a truly periclinal fashion, whereas the two lateral ones divide periclinally or somewhat obliquely. Continuous periclinal activity of these cells gives rise to



Figs. 1–7. *Liriodendron tulipifera*. pl = placenta; ov pr = ovule primordium; ii = inner integument; oi = outer integument; nuc = nucellus. Figs. 1, 2. Cross sections of carpels showing 3-zonate placental regions. – Figs. 3, 4. Details of placentae with ovule initiation. – Figs. 5–7. Ovules showing dermal initiation of inner integument and subdermal initiation of outer integument.



Figs. 8–13. *Liriodendron tulipifera*. nc = nucellar cup; pc = parietal cells; pv = provascular tissue. Figs. 8, 9. Longitudinal sections of ovules. – Figs. 10–13. Cross sections of ovules and young seeds showing early phases of seed coat formation.

a three, sometimes locally four-layered i.i. (figs. 6, 7 and 8). After having reached the apex of the nucellus, the i.i. becomes multi-layered in its apical part to form the endostome. The endostome is more or less biapiculate, with its slit situated parallel with the raphe (figs. 11b and 11c).

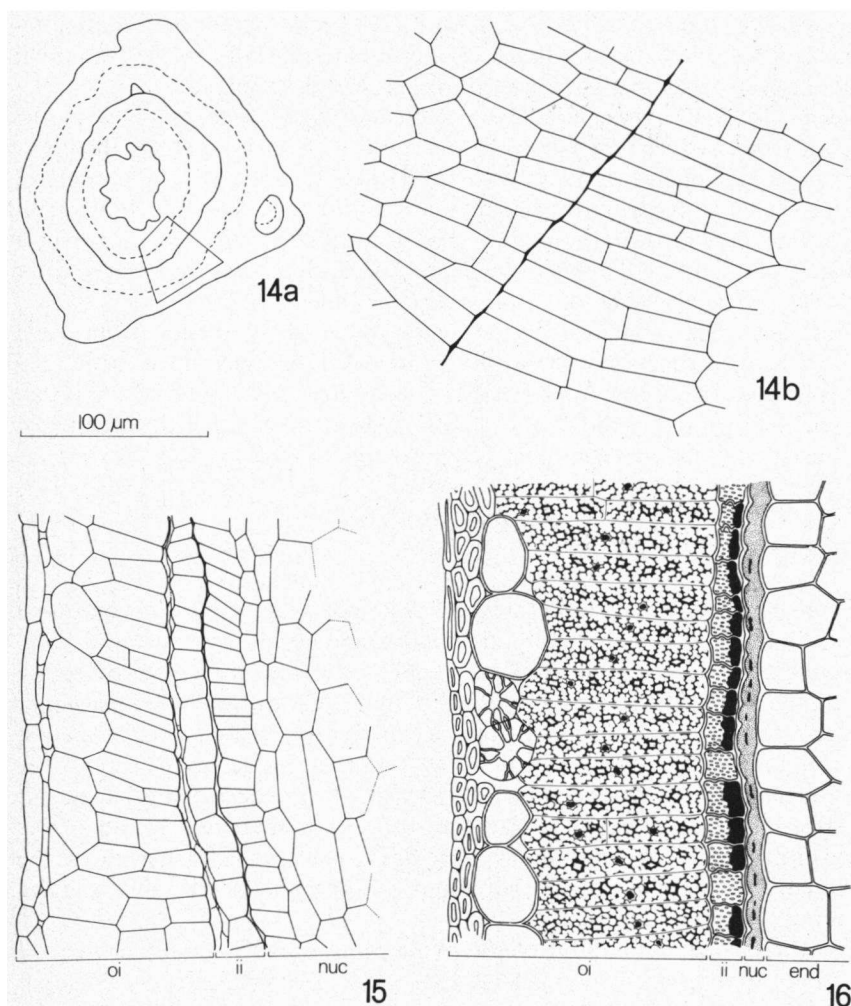
Shortly after the initiation of the i.i., the outer integument (o.i.) originates by periclinal divisions of the second tunica layer. The initiation starts first at the convex side of the ovule (figs. 5 and 6). However, during its later ontogenetic stages, the o.i. is clearly discernible at the raphal side (figs. 7 and 8). The outer dermal layer of the o.i. remains one cell thick, except in its apical part which forms a small dermal cap. The o.i. overgrows the i.i. only at its lateral flanks. The o.i. is initially only three- to four-layered in cross section. However, already during megagametogenesis the subdermal tissue at the flanks of the o.i. starts dividing and forms two lateral ridges running from the chalaza up to the micropyle. Within each ridge lies a strand of small provascular-like cells, but differentiated vascular elements could not be observed, however (fig. 12).

The ovules are innervated by the ventral bundles of the carpel, which in their turn are connected with the outer ring of bundles of the floral axis. The dorsal bundle is derived from the inner ring of the floral axis and is clearly differentiated before the ventrals. The funicle and raphe contain a collateral bundle, the phloem lying peripherally. This bundle shows a well-developed cambial zone and differentiated xylem and phloem elements. The raphe bundle bifurcates near the chalaza. The larger bundle enters the chalazal tissue between o.i. and i.i. and ends in a dendroid ramification, mingling with the tannin-containing tissue of the hypostase. The smaller branch ramifies and ends at the base of the o.i., two of its smaller offshoots being directed towards the two lateral ridges of the o.i.

During seed formation, the massive nucellar tissue is gradually resorbed starting from the inside by the growing endosperm. In the mature seed coat only its epidermis and remnants of adjacent subdermal cells are discernible (compare figs. 10, 12, 13, 15 and 16). Between the i.i. and the nucellus is a distinct cuticle.

Also after fertilisation the i.i. does not increase in thickness and remains mostly three-layered. The outermost two layers first keep up with the increase in girth of the developing seed by anticlinal divisions, but later on the cells become extended, flattened and crushed. Remnants of these cells are present in the mature seed coat only locally. The cells of the innermost layer of the i.i. differ by their size and their tanniferous contents and by their persistence also during the later stages of seed development (figs. 15 and 16).

The cells of the outer epidermis of the o.i. contain tannin, become tangentially elongated and differentiate into a layer of fibrous, sclerotic cells with lignified walls during the later phases of seed maturation. The thickness of the subdermal tissue of the o.i. clearly differs in the anti-raphe part, in the ridge and in the part between the ridge and the raphe (fig. 12). At the anti-raphe side only two or three layers of subdermal cells are present. They first acquire a more or



Figs. 14–16. *Liriodendron tulipifera*. end = endosperm. Fig. 14. Cross section of young seed between attachment of inner and outer integument showing two sclerotic layers facing one another. – Fig. 15. Cross section showing periclinal divisions of inner epidermis of o.i., compression of outer layers of the i.i., and resorption of the nucellus. – Fig. 16. Cross section of the mature seed coat.

less parenchymatic appearance, by forming small intercellular spaces. The cells joining the outer epidermis become irregular in shape and more or less elongated. They differentiate into sclerotic cells with lignified, pitted cell walls. The cells joining the inner epidermis, to the contrary, become larger and remain isodiametric. They develop partly into vacuolised, parenchymatic cells, partly into sclereids with thick, lignified walls with branched pit canals. The cells of the inner epidermis of the o.i. are rich in cytoplasm, become radially stretched

and may divide once or twice (figs. 13 and 15) to form one or two, sometimes three layers of sclereids, with thick, labyrinth-like cell walls. In the remaining protoplast crystals and a degenerated nucleus can be observed.

During seed development a clear intercalary growth takes place in the chalazal tissue between the bases of the i.i. and o.i. which separates the insertion of the integuments from one another. The epidermal layer of this chalazal tissue behaves similarly to the inner epidermis of the o.i. The cells show radial elongation, periclinal divisions and sclerification (fig. 14b), thus forming a tubular sheath: the heteropyle of the mature seed. Sometimes a temporary air space between chalaza and o.i. is present (figs. 9 and 14a).

There are small differences in the development of the ovules within each flower. The development of the ovules and seeds of the uppermost carpels lag a little behind that of the lower ones. The seeds grow to a length of 4 to 5 mm and remain enclosed in the fruit wall. The endosperm is copious, the embryo minute, straight, and lies near the micropylar end.

4. DISCUSSION

The ovule primordium of *Liriodendron* is described as three-zonate, composed of two, initially only anticlinally dividing, layers enveloping a central core. Although no details are known of the change-over from the vegetative to the floral apex and of the initiation of the different floral organs in *Liriodendron*, FOSTER's (1950) conclusion that the vegetative apex of all woody Ranales, including *Liriodendron*, investigated by him are characterised by a two-layered tunica (see also GUNCKEL 1950 and HAGEMANN 1960), renders it plausible that the three-zonate structure of the placental region and of the ovule primordium are continuous with the corpus-tunica structure of the vegetative apex, in other words, that it may be interpreted as two tunica layers enveloping a corpus.

This three-zonate condition of the ovule primordium is of rather general occurrence among angiosperms (BOUMAN 1974). According to my personal observations it is found in *Magnolia* and *Drimys*, and in taxa belonging to the Ranunculaceae, Berberidaceae, Papaveraceae, Staphyleaceae, Cistaceae, Caricaceae, Cruciferae, Capparidaceae, Resedaceae, Geraniaceae, Rutaceae, Malpighiaceae, Cucurbitaceae, Sterculiaceae, Malvaceae, Euphorbiaceae, Thymelaeaceae, Bignoniaceae, Convolvulaceae, Boraginaceae, Scrophulariaceae, Solanaceae and Liliaceae (see also WARMING 1878: Grossulariaceae, Violaceae and Scrophulariaceae, SATINA 1945: Solanaceae, and KORDYUM 1968: Umbelliferae, Casuarinaceae, Rosaceae, Betulaceae, Polygonaceae, Nyctaginaceae, Vitaceae, Plumbaginaceae, Amaryllidaceae and other ones).

The wide-spread occurrence within the angiosperms, both in families of a supposedly primitive status and in derived families, may lead to the conclusion that the three-zonate ovule primordium is basic in angiosperms. This clashes with the classical carpel concept, in which the carpel is interpreted as a meta-

morphosed leaf bearing ovules on the margins or on the adaxial surface of the lamina. Leaf primordia may have a three- or two-zonate structure. However, also in the case of a three-zonate leaf primordium, the central component, being in the great majority of cases the corpus, forms a part of the petiole and midrib only, the leaf lamina being formed exclusively by the two outermost zones.

Magnolia and *Liriodendron* show a great similarity in their ovule ontogeny and in the initial phases of seed development. Both taxa possess three-zonate ovule primordia, nucelli with massive parietal tissue, a 3-layered, dermal inner integument, and a subdermally initiated outer integument. In both taxa the inner integument does not play any important role in seed coat formation, and the nucellus, except for its epidermis, is resorbed by the endosperm. In both genera an intercalary growth takes place in the chalazal region between the integuments and a hypostase is present. The main differences lie in the differentiation of the outer integument. The outer integument in *Liriodendron* develops two small lateral ridges, each with a strand of provascular-like tissue, whereas in *Magnolia* the bilateral symmetry of the outer integument is only reflected in its vascularization pattern and in the exostome. In both genera the inner epidermis of the outer integument shows a radial elongation, followed by periclinal divisions and a differentiation into the characteristic sclerotesta (endotesta sensu Corner). The development of the subdermal tissue of the o.i. is altogether different. In *Magnolia* this tissue becomes multicellular and develops into a parenchymatous sarcotesta, whereas in *Liriodendron* it remains thin-layered and becomes partly sclerotic. The ultimate differences in seed coat structure of *Magnolia* and *Liriodendron* are clearly associated with the differences in seed dispersal.

This study is in agreement with Dandy's subdivision of the family and confirms the general opinion, not only based on phytogeographical but also on morphological, palynological, and anatomical evidence, that *Liriodendron* is the most advanced genus in the Magnoliaceae (see BARANOVA 1972, GOTTWALD 1975, DANDY 1967, TAKHTAJAN 1969, WALKER 1976).

The obturator said to occur in *Liriodendron* by KAEISER & BOYCE (1962) is nothing but the part of the outer integument at the raphe side.

As regards Zazhurilo's and Takhtajan's typification of the magnolialean seed coats it may be concluded that their proposed types and semophyletic relations may be acceptable (the more so in the light of KAPIL & JALAN's (1964) finding that in *Schizandra grandiflora* few of the cells of the inner epidermis of the outer integument undergo sclerification), but their conclusions do not reflect all major evolutionary trends. Their classification is in fact mainly based on the topography of the parenchymatic and sclerenchymatic tissues of the mature seed. Moreover, seed coat formation in the Magnoliales is complicated by such processes as rumination (Degeneriaceae, Annonaceae, Myristicaceae, Canellaceae), chalazal growth (Myristicaceae), the formation of arils (Annonaceae, Myristicaceae), and the change-over to a dermal initiation of the outer integument (Winteraceae, Lactoridaceae). Additional detailed

information regarding seed coat development in the magnoliids is urgently required.

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