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# INTEGUMENTARY STUDIES IN THE POLYCARPICAE III. DRIMYS WINTERI (WINTERACEAE)

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SUMMARY

*Drimys winteri* has an anatropous, crassinucellate type of ovule with two non-vascularized integuments. Both the inner integument (i.i.) and the outer one (o.i.) are of dermal derivation. The integuments consist of three, or locally sometimes two or four layers of cells. The dermal initiation of the o.i. is in marked contrast with the subdermally initiated o.i. of the Magnoliaceae. This difference in origin of the o.i. is an additional character justifying the dissociation of the Winteraceae from the Magnoliaceae.

## 1. INTRODUCTION

Our knowledge of the initiation and growth of the ovular integuments is still scanty and these phenomena have been studied in greater detail in a few taxa only: ROTH 1957 (*Capsella*), BOESEWINKEL & BOUMAN 1967 (*Juglans* and *Pterocarya*), BOUMAN 1971a (*Lilium*), 1971 b (*Lactoris*), and DE BOER & BOUMAN 1972 (*Magnolia*). In the majority of the embryological papers the authors only mention the sequence of initiation of i.i. and o.i., the number of cell layers of each integument, the contribution of the integuments to the formation of the micropyle, and the presence or absence of tegumentary vascular bundles. The mode of initiation of the integument(s), i.e., a dermal or a subdermal initiation, and the pattern of growth are neglected aspects. The present authors are of the opinion that integument initiation and development could well be taxonomically and phylogenetically applicable features and therefore deserve further study. The Winteraceae, as one of the vesselless taxa among the Dicotyledons, have interested taxonomists and phylogeneticists for a considerable length of time. The embryology of the Winteraceae has been studied relatively well: SWAMY 1952 (*Zygogynum*), BHANDARI 1963 (*Pseudowintera*), SAMPSON 1963 (*Pseudowintera*), BHANDARI & VENKATARAMAN 1968 (*Drimys*). None of these workers gives any detailed information concerning integument initiation, however.

Sampson said about *Pseudowintera:* "The integuments are initiated while the megaspore mother cell is enlarging, when the ovule has undergone inversion of about 30°. It would seem from the shape and size of young integuments that they are initiated almost simultaneously".

In his publication the thickness of the i.i. is reported to be two to three cell layers, and of the o.i. three cell layers. In the micropylar part the i.i. may become multilayered. BHANDARI (1963) recorded for *Pseudowintera:* "The o.i. is shorter and thicker, while the inner projects beyond it to form the micropyle". As regards the ovular vascularization he stated: "The funicular trace ends at the base of the chalaza in each ovule", thus implying that the integuments are not vascularized. In all three genera previously studied tannin-like compounds are already present in both integuments and in the dermal and subdermal cells of the funicle in an early phase of ovular development. In the present study the earlier observations concerning ovular structure of *Drimys winteri* were confirmed and extended by an investigation of the initiation and growth pattern of its integuments.

## 2. MATERIALS AND METHODS

All the material used in the present study was obtained from a single specimen cultivated in the Botanical Garden, Amsterdam. This plant flowers regularly in January/February every year, but unfortunately fruit setting does not take place even after artificial pollination (self-incompatibility?). Young flowers or isolated gynoecia of older buds and open flowers were fixed in Craf III and IV, dehydrated by means of a t.b.a. series, and embedded in paraplast. Sections were cut 7 μm thick and these were stained with safranin (according to Johanson) counterstained with astra blue. Owing to the rather inconstant orientation of the ovules in the ovary only a relatively small number of ovules was cut exactly along the median plane and suitable for developmental studies. Camera lucida drawings were made of selected sections.

## 3. OBSERVATIONS

### 3.1. Generalities

The placenta forms a U- or ring-shaped zone around the pistillar cleft (see *fig. 1*). The ovules face each other with their raphal sides. Each follicle contains about 13 to 16 ovules. The ovules of one pistil develop almost simultaneously, but the uppermost ones may lag a little behind in development. The ovules are anatro-

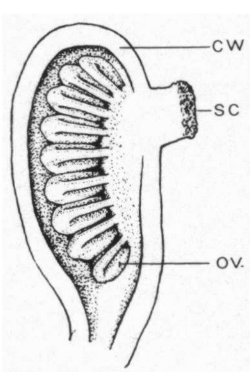



Fig. 1. Longitudinally cut carpel, seen from the inside, showing the arrangement and orientation of the ovules. cw = carpel wall, ov. = ovule, sc = stigmatic crest.

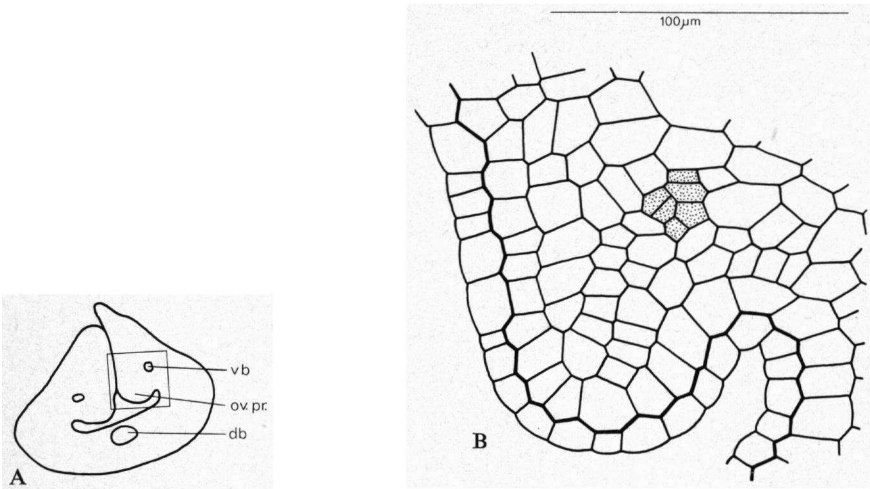



Fig. 2. A: Transverse section of a carpel with ovule primordia. ov.pr. = ovule primordium, db = dorsa bundle, vb = ventral bundle.
B: Detail of *fig. 2 A*, showing 3-zonated ovule primordium.

pous, bitegmic and crassinucellate. The ovules are initiated subdermally, as is the fixed rule in Angiosperms. The ovule primordium shows three zones in a median l.s. (*fig. 2*). There is one single-layered dermal zone where cell-division is anticlinal only, except in the integument initials and in the apical cells of the young nucellus where during gametogenesis a small nucellar cap will be formed. The subdermal zone is initially one cell layer thick in which at an early stage already an archesporial cell becomes differentiated, the latter subsequently forming, by means of periclinal divisions, the primary sporogenous cell and the parietal tissue. All other cells of this layer also divide periclinally, which results in an increase in girth of the nucellus and in a broadening of the chalaza. The third, central zone is multicellular and responsible for the growth in length of the ovule; it partly differentiates into the procambial strand of the future funicular trace. In accordance with the ontogenetic studies of *Drimys winteri* flowers by TUCKER (1959), this stratification is not to be interpreted as two tunica layers enveloping a corpus. As far as the second and third zone are concerned, this zonation must be of a secondary nature (i.e., it is not directly related with the structural pattern of the floral and the shoot apex and of the epicotyl primordium). A stronger rate of growth at the convex side of the ovule primordium sets in the curvation ultimately leading to the anatropous condition of the ovule (*fig. 3*). The inner integument (i.i.) is initiated earlier than the outer integument (o.i.). The latter develops only on the dorsal and the lateral sides of the ovule: in a median l.s. there is no sign of the o.i. at the funicular side (*fig. 3*). The i.i. and o.i. initials are in close contact. Below the zone of insertion of the integuments not infrequently some periclinal divisions can be observed in the subdermal zone (*figs. 4* and *8*); if this is the case the integu-

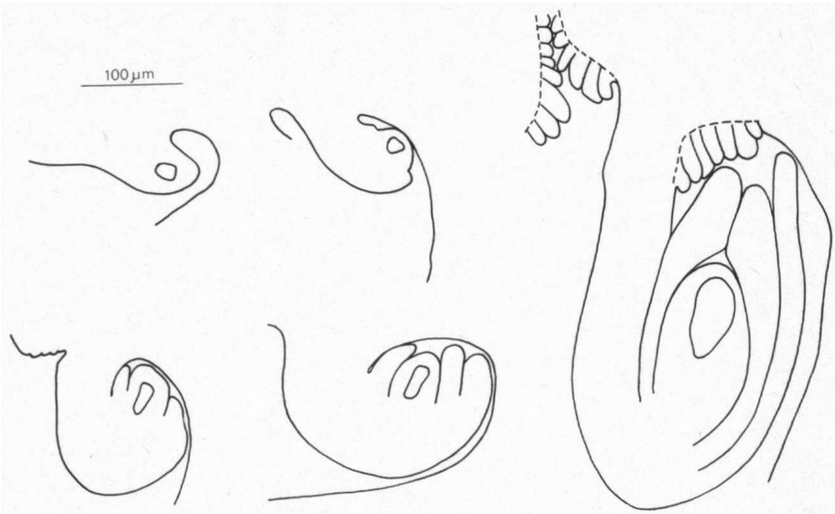



Fig. 3. Longitudinal sections of ovules in different stages of development.

ments become somewhat raised. Both integuments are of dermal origin and do not exhibit any appreciable differences in initial development.

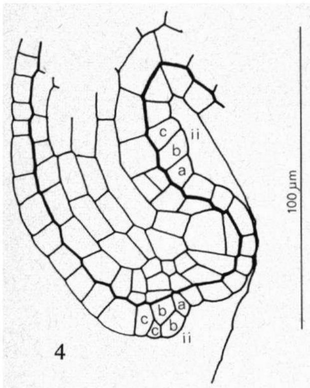
The epidermal cells of the stigmatic crest, the interlocking cells of the pistil cleft, the epidermal cells of the placentae, and those of the basal part of the funicle are more or less papillose and most probably play a part in the conduction of the pollen tubes.

The i.i. forms a narrow endostome. The o.i. overgrows the i.i. to some extent, but it does not form a closed exostome; the space between the free rim of the o.i. and the funicle is partly filled in by the papillose epidermal cells of the placenta and funicle base. Cells containing deeply staining polyphenolic compounds are mainly located in the i.i. and o.i., but are also present in the subdermal and dermal cells of the funicle (and the raphe).

The funicle contains an open, collateral vascular bundle, the xylem part of which is situated – as is normally the case – at the concave side of the ovule, the phloem part at the convex side. The xylem forms well developed elements with spiral wall thickenings. The bundle sometimes divides in the chalaza. The integuments are not vascularized.

## 3.2. The inner integument

The first indication of the initiation of the i.i. is noticeable as a slight swelling of a dermal cell, or of several such cells (*fig. 4*). In these swollen cells soon periclinal division walls appear (*figs. 4* and *5*). As stated previously, also subdermal cells situated below the integument primordium exhibit mitotic activity, especially at the dorsal side of the ovule primordium, thus slightly raising the base of the integument (*figs. 4* and *8*). In a median section usually three dermal cells exhibiting periclinal cell division can be observed. These three cells and



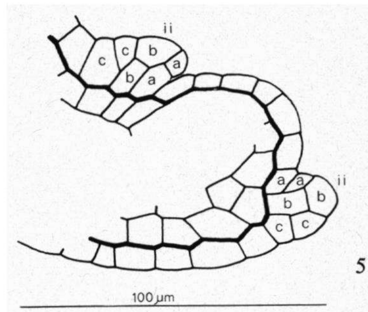




Fig. 4. Longitudinal section of an ovule with first indication of the initiation of the inner integument. (i i)
Fig. 5. Longitudinal section of an ovule, somewhat older stage than the one shown in *fig. 4*, with periclinal divisions of the dermal initials of the i i.

their derivatives – together forming the integument primordium – are indicated in the figures by the letters a, b, and c (*figs 4* and *5*). The middle one (cell b) usually divides in a truly periclinal fashion, whereas the two lateral ones (cells a and c) often divide by means of a somewhat oblique division wall situated between the common lateral wall of the mother cell and the central initial and the outer wall (cell c in *fig. 4*, cells a and c in *fig. 5*). This oblique wall is usually oriented in such a way that the more highly situated daughter cell is as it were deposited against the middle initial.

During the subsequent growth of the integument primordium the three initials a, b, and c each produce a row of cells, so that an integument originates which appears to be three-layered in a transverse section (*fig. 6*: i.i. at the dorsal side). If the cell (or cell row) a or c lags somewhat behind in mitotic activity, a situation may develop in which the integument is two-layered (*fig. 6* i.i. at the funicular face, *fig. 8*). All cells and their derivatives retain their mitotic activity for a considerable length of time: growth by means of an apical cell does not take place. The integument is kept pressed against the nucellus by a somewhat stronger mitotic and/or stretching activity of the peripheral cells. In later stages of development of the ovule the cellular pattern can not be so easily interpreted any longer, mainly because of the increased mitotic activity in the micropylar part of the integument. During and after the closure of the endostome both the nucellus and the integument exhibit an appreciable growth. The three cell layers of the i.i. still retain their identity (*figs. 9* and *10*). Around the endostome the i.i. becomes multilayered by divisional walls oriented parallel to the surface of the integument. In the full-grown ovule the cells of the inner layer contain an appreciable quantity of tanniferous cells containing polyphenolic compounds (*figs. 9* and *10*); such tanniferous cells also occur scattered in the micropylar part of the i.i.

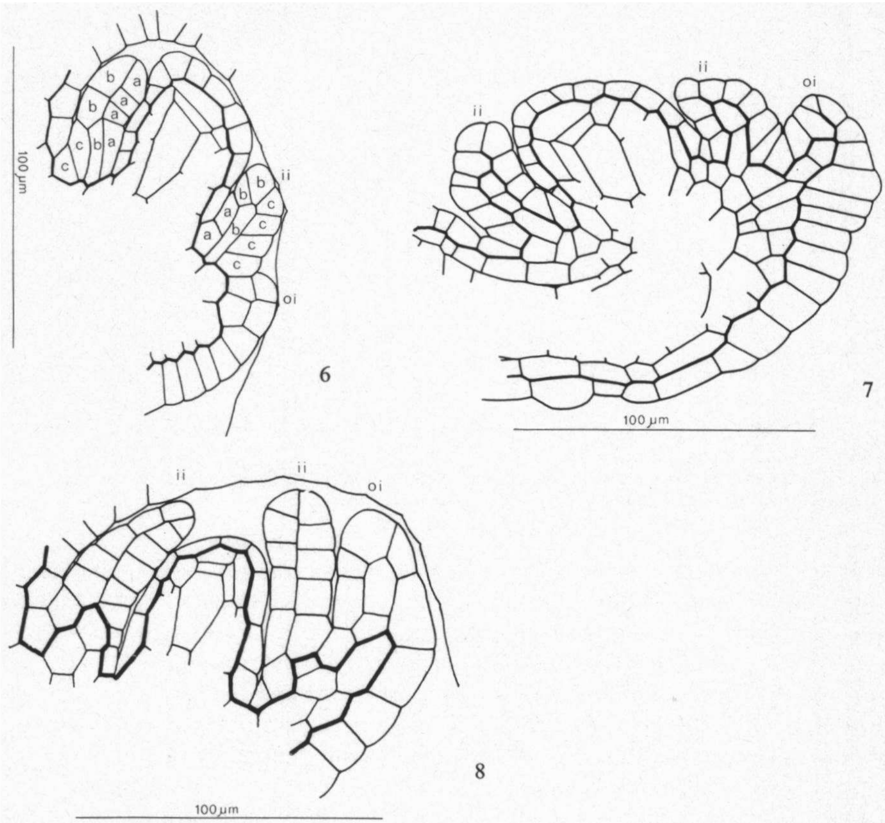



Fig. 6, 7, 8. Longitudinal section of an ovule, with successive developmental stages of the outer integument. i i = inner integument, o i = outer integument.

## 3.3. The outer integument

The initiation of the o.i. commences a short time after that of the i.i. Periclinal cell divisions occur in three (or sometimes two) adjacent dermal cells immediately adjoining the i.i. primordium (*figs. 6* and *7*). In the same way as in the i.i. initiation, each of these three (or occasionally two) initials furnishes one of the (normally) three cell layers forming the o.i. The cells and their derivatives also remain meristematic and there is no growth by means of an apical cell, either. Owing to a slightly higher rate of mitotic activity of the outer layer the o.i. is pressed against the i.i. (*fig. 7*).

The o.i. is not developed at the funicular side. The o.i. apically overgrows and overtops the i.i. to some extent, but it does not form a closed exostome. As a rule it remains three-layered throughout (*figs. 9* and *10*). The outer cell layer becomes packed with tannin (*fig. 10*).

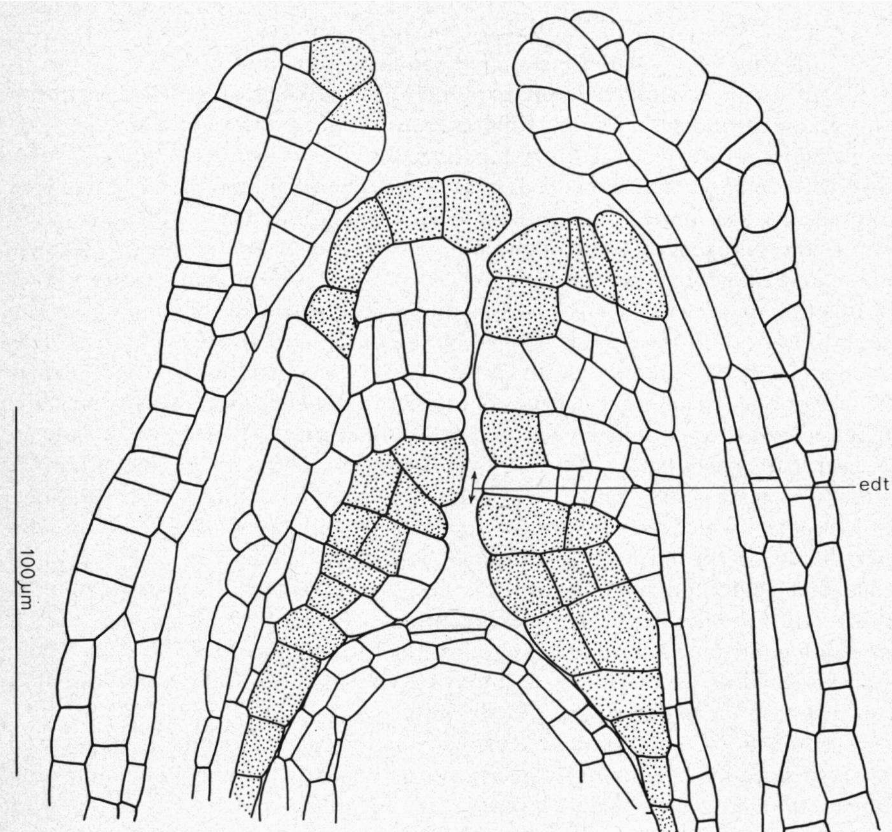



Fig. 9. Transmedian l.s. of the micropylar portion of a mature ovule. Stippled: cells with polyphenolic compounds. edt = endostome.

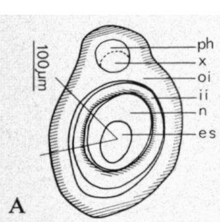
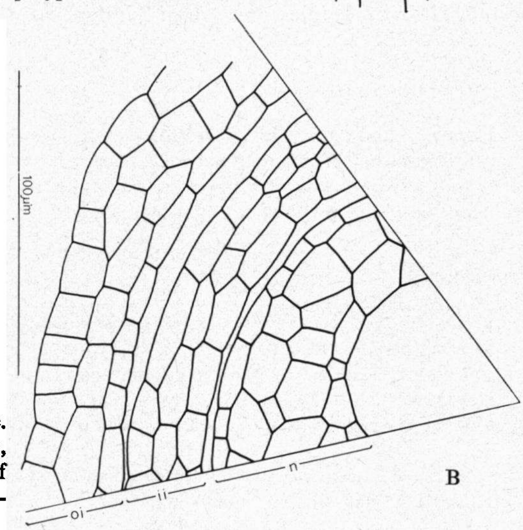



Fig. 10. A: T.s. of a mature ovule. es = embryo sac, n = nucellus, x = xylem, ph = phloem. B: Detail of *fig. 10 A*. Hatched: cells with polyphenolic compounds.



## 4. DISCUSSION

Although our limited knowledge of integument initiation in the Angiosperms does not permit a detailed comparison between various taxa, one salient result was obtained: there is a striking difference in integument initiation and development between Winteraceae and Magnoliaceae. The Winteraceae have a three-layered, non-vascularized o.i. of dermal origin, the Magnoliaceae a multilayered and usually vascularized o.i. with subdermal initiation. These differences constitute the ontogenetic basis of the different seed coat architecture in Winteraceae and Magnoliaceae already known from several published reports.

In Winteraceae each pistil develops into a berry-like fruit containing several seeds. The bulk of the testa is formed by the outer layer of the o.i., whose cells elongate radially and become thick-walled, very hard and packed with phenolic compounds. The other cell layers remain thin-walled and usually become much flattened or completely crushed at seed maturity. All cell layers of the i.i. become squashed flat (SWAMY 1952; BHANDARI 1963, 1971; BHANDARI & VENKATARAMAN 1968; VINK 1970). This is in marked contrast with the Magnoliaceae in which the innermost (epidermal) cell layer of the o.i. divides periclinally to form a hard sclerotesta, the central (subdermal) cell layers and the outer epidermal layer becoming a fleshy sarcotesta with essential oil cells (except in *Liriodendron* which has indehiscent fruits). In both families the i.i. does not contribute substantially towards the formation of the seed coat. These well-known differences in testa architecture appear to be founded on essential differences in the initiation and growth of the o.i.

In addition to the anatomical and morphological arguments of Bailey and his associates and the embryological data adduced by Swamy, Bhandari, and others, the ontogenetic analyses support the segregation of *Drimys* and its near allies a sa separate family, Winteraceae, rather than the merging of these taxa with the Magnoliaceae as was done in the older systems of classification (Bentham & Hooker, Engler, Wettstein, Hallier, etc.)

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