

OVULE ONTOGENY AND SEED COAT DEVELOPMENT IN GENTIANA, WITH A DISCUSSION ON THE EVOLUTIONARY ORIGIN OF THE SINGLE INTEGUMENT

F. BOUMAN and S. SCHIER

Hugo de Vries Laboratorium, Universiteit van Amsterdam, Plantage Middenlaan 2A, 1018 DD Amsterdam

SUMMARY

In *Gentiana* both dermal and subdermal cells are involved in integument formation. The sequence and positions of the dermal and subdermal initials are reminiscent of the situation in bitegmic ovules and render it plausible that this single integument is derived from a dermal inner and a subdermal outer integument by the process of integumentary shifting. This study shows that the diversity in integument initiation in the sympetalous taxa is greater than is generally assumed.

1. INTRODUCTION

The structure of the ovule and the mature seed in such sympetalous taxa as Gentiananae, Asteranae and Lamianae is, generally speaking, rather simple (see the handbooks of SCHNARF 1931, DAVIS 1966, NETOLITZKY 1926 and CORNER 1976).

All the families included are tenuinucellate. Only in certain species of the Convolvulaceae, Polemoniaceae, Boraginaceae and Caprifoliaceae have crassinucellate ovules been recorded.

The nucellus is always resorbed at an early stage and thus does not contribute to the formation of the seed coat.

All the families are unitegmic. And in the majority of cases only the outer layer of the integument differentiates into an anatomically characteristic structure, whereas the other layers of the integument become compressed and are later fully or partially resorbed. In a minority of the families its middle and/or inner layer can still be recognised in the mature seed coat, but only in a small number of taxa as clearly differentiated cell layers.

In this respect the most characteristic example is provided by the seed coat of the Convolvulaceae and the closely related Cuscutaceae. They differ from all other unitegmic seeds of the Sympetalae by the presence of three differentiated outer cell layers: an epidermal, a hypodermal and a palisade layer. Of these the palisade layer is the most prominent sclerotic one. It consists of radially elongated cells with thickened walls, often provided with a "light-line". The hypodermal and palisade layer may be one- to pluri-layered and have a common derivation from the subdermal layer of the integument. This subdermal layer is clearly discernible from the dermatogen throughout the ovule ontogeny (see,

e.g., SCRIPLENG & SMITH 1960, KAUR & SINGH 1970, GOVIL 1970, and personal observations). CORNER's (1976) interpretation of the three layers as a multiplicative epidermis, and consequently also his comparison of the seed coats of the completely unrelated *Convolvulaceae*, *Cucurbitaceae* and *Paeoniaceae*, is incorrect. There are no other unitegmic sympetalous families in which the differentiation of hypodermal seed coat layers is a general characteristic.

An example of the differentiation of the middle layer of the single integument at the generic level is provided by *Menyanthes* (GUÉRIN 1904, MAHESHWARI DEVI 1962). Here the parenchymatic middle layer develops intercellular spaces and pitted, thickened cell walls. At maturity its cells become filled with air and are responsible for the floating capacity of the seed. Similar tissues are found in other water plants with floating seeds. In the other genera of the *Menyanthaceae* the epidermal layer and its derivatives are mainly responsible for the drifting capacity; the middle layer remains parenchymatic and becomes partly compressed and resorbed. Specializations within the cells of the middle layer, often in the form of reticulately thickened walls, have also been recorded in certain genera of the *Acanthaceae*, *Apocynaceae*, *Bignoniaceae*, *Loganiaceae*, *Martyniaceae*, *Polemoniaceae*, *Solanaceae* and *Verbenaceae*.

The development of the innermost layer of the integument into an endothelium during gametogenesis, or during the early stages of seed development, is a general characteristic of many sympetalous families, especially within the *Dipsacales*, *Ericales*, *Scrophulariales* and *Solanales* alliances. In many families the endothelium disintegrates and subsequently disappears during the later phases of seed development. Only in some of these families does the endothelial layer remain distinct in the mature seed coat, for instance in *Polemoniaceae* and *Plantaginaceae*, where it forms a pigment layer, and in the *Lentibulariaceae*, *Scrophulariaceae* and *Solanaceae*.

It is clear that most of the unitegmic sympetalous families have seeds which can be called exotestal in the terminology of Corner. This uniformity is in great contrast with the bitegmic angiosperms in which, on the basis of the position of the main mechanical layer, Corner distinguishes six principal types, namely exo-, meso- and endotestal, and exo-, meso- and endotegmic seeds. The small number of pages (10 out of the 552) of Corner's second volume which are dedicated to unitegmic and sympetalous families is significant of the disinterest.

From all the above-mentioned data it will be clear that the characters of ovule and seed as far as known to date can be of only relatively little significance in discussions about the phylogenetic interrelationships of the various sympetalous families. In practice the outer layer of the seed coat provides the main diagnostic characters. The great diversity in cell shape and of the cell wall thickenings can be of great help at the specific and generic level, but fails as a marker of the evolutionary lines at the family and order levels.

In spite of this rather negative conclusions there may be some hope that further and more integrated studies on ovule and seed coat characters in the unitegmic ovules may yield additional indications. Detailed studies of the ovule

and integument initiation in sympetalous families are very scanty. Most data still date from WARMING (1874). He observed that ovules of *Lamium*, *Symphytum* and *Verbascum* are initiated in the third layer of the placenta, whereas the ovules of *Senecio*, *Gesneria*, *Lobelia* and *Monotropa* are formed by divisions of the subdermal layer. As regards the integument in unitegmic ovules he stated that in spite of its thickness, it is mainly of dermal derivation in *Symphytum*, *Lamium*, *Verbascum*, *Odontites*, *Syringa*, *Lonicera*, *Menyanthes*, *Jasione*, *Lobelia*, *Eccremocarpus* and *Gesneria*. Independent evidence of the dermal origin of the integument was obtained by SATINA (1945) from observations on cytochimaeras of *Datura*. BHANDARI, BOUMAN & NATESH (1976) described the three-zonate nature of the ovule primordium and the dermally initiated integument of *Scrophularia himalensis*.

The lack of data on integument initiation in unitegmic ovules renders discussions concerning the way (or ways) in which the sympetalous families have become unitegmic more speculative than is necessary. This study demonstrates that ovule and integument initiation can be more complicated than is generally assumed.

2. MATERIAL AND METHODS

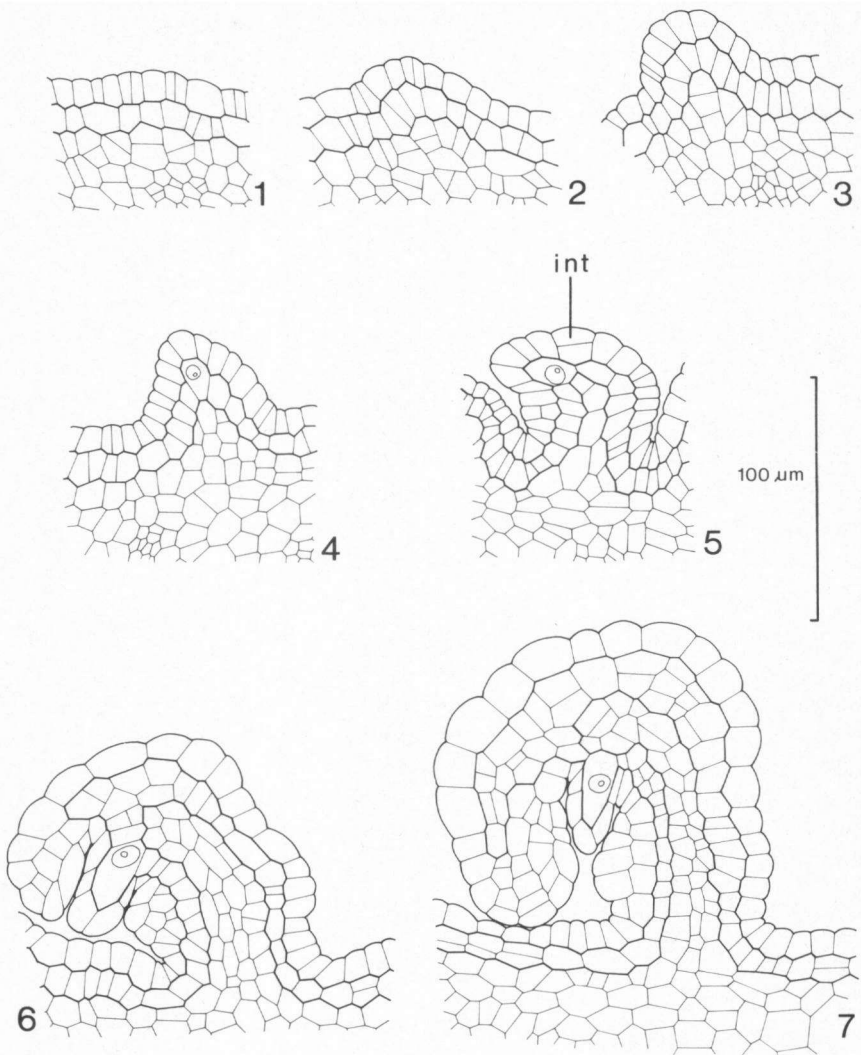
Series of developmental stages of *Gentiana cruciata* L. and *Gentiana asclepiadea* L. were collected from plants of the Botanic Garden of the University of Amsterdam. The material was fixed in CRAF and Allen-Bouin mixtures, dehydrated in a TBA-series, embedded in paraplast, sectioned at 7 μ m, and stained in a safranin-astra blue combination. Mature seeds were embedded in epon and also sectioned by hand for specific staining with phloroglucinol, aniline sulphate, Ruthenium red and Sudan IV. SEM photographs were taken with the aid of a Cambridge Stereoscan Mark 2a.

3. OBSERVATIONS

3.1. *Gentiana cruciata*

The placenta shows a clearly 3-zonate structure (*fig. 1*). The ovules are initiated by periclinal divisions in the third zone (*figs. 2, 3 and 4*), the cells of the dermal and subdermal layer multiplying exclusively by anticlinal divisions. Very soon an archesporial cell differentiates at the top of the ovule primordium and the anatropous curvation commences. The integument is initiated by periclinal and oblique divisions in two or three dermal cells (*fig. 5*). Continued divisions of these initials give rise to an integument which remains dermal until it reaches to the top of the nucellus (*fig. 6*). From this moment onward the subdermal cells lying below and somewhat proximally in relation to the integument primordium start dividing periclinally. By this mitotic activity the dermal cell rows shift towards the micropylar part of the integument (*figs. 7 and 8*).

The ovule is unitegmic and tenuinucellate, and the integument forms a long

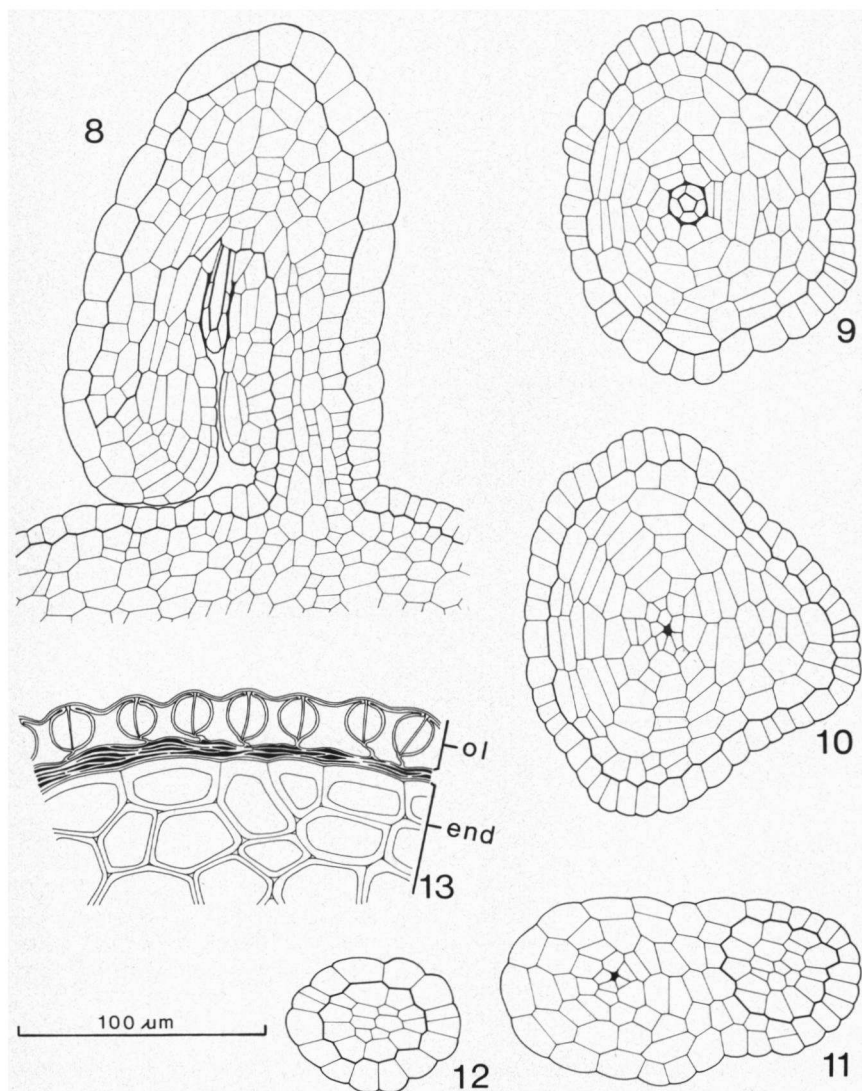


Figs. 1-7. *Gentiana cruciata*. Longitudinal sections of ovule primordia and young ovules showing ovule initiation, integument initiation and anatropous curvature. int = integument.

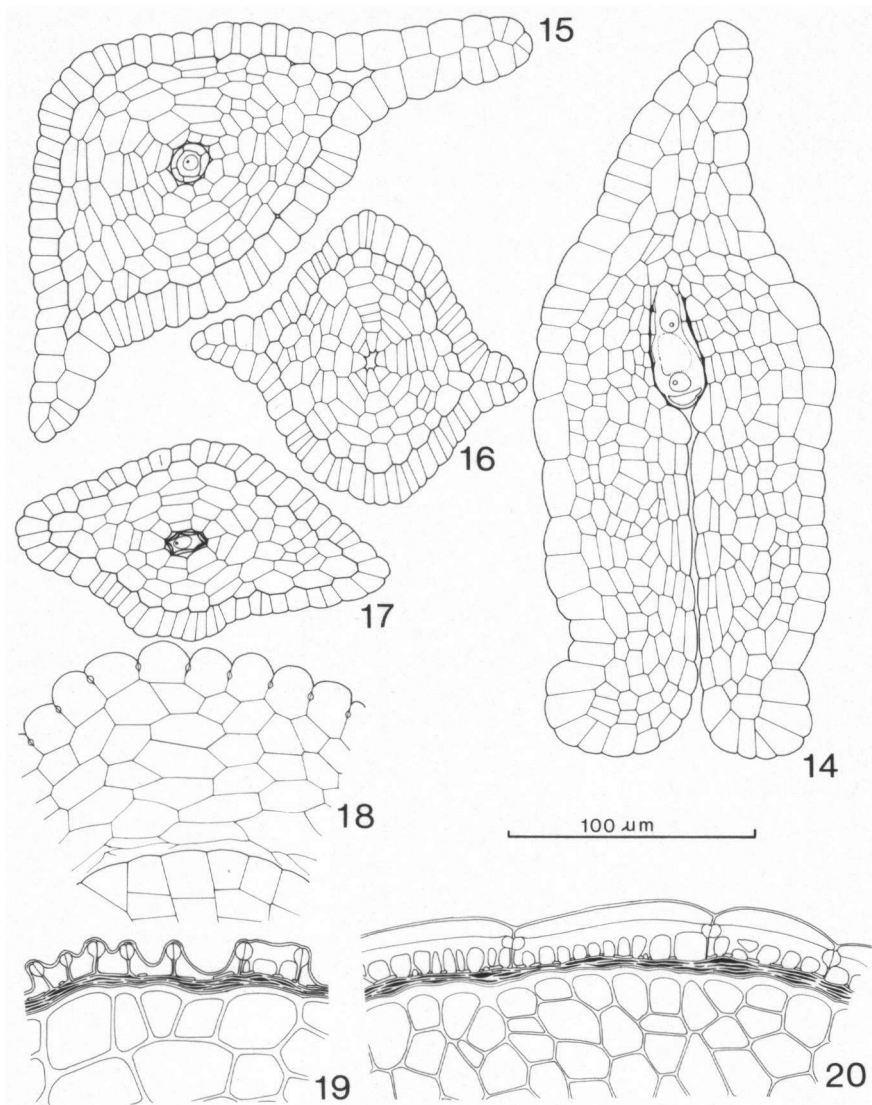
micropyle. The nucellus is small and consists of the derivatives of the arche-sporial cell only surrounded by a nucellar epidermis. The integument is 6 to 10 cells thick and does not form an endothelial layer. The vascular supply of the ovule is almost completely reduced. In the raphe and funicle smaller cells can be observed, which do not differ by their protoplasmatic contents or otherwise, and do not form a distinct procambial tissue (figs. 9-12).

The seed coat formation is rather simple. The number of cells of the surface of the seed hardly exceeds that of the ovule. Of the integument only the outer layer

differentiates. Its cells contain tannin and stretch longitudinally. Especially the cells along the raphe have very narrow lumina (*photogr. 2 and 6*). On the anticlinal cell walls hemispheric, lignified thickenings are formed (*fig. 13*). The two halves of two adjacent cells together form annular bars, which determine the more or less reticulate pattern of the seed surface (*photogr. 1-6*). The tangential cell walls do not show any thickenings. The outer cell walls are covered by a



Figs. 8-13. *Gentiana cruciata*. Fig. 8. Longitudinal section of ovule with closed micropyle. Figs. 9-11. Cross sections at the levels of the nucellus and micropyle. Fig. 12. Cross section of the funicle. Fig. 13. Cross section of the mature seed coat. ol = outer layer; end = endosperm.



Figs. 14–20. *Gentiana asclepiadea*. Fig. 14. Transmedian longitudinal section of ovule with young embryo sac. Figs. 15–17. Cross sections of ovules at different levels showing seed wing. Fig. 18. Cross section of a developing seed coat. Figs. 19–20. Cross and longitudinal sections of mature seed coats.

cuticle and are in the dry, mature seed bent inwards. All the other, tegumentary layers become resorbed or compressed.

The endosperm occupies the greater part of the seed and is separated from the seed coat by a cuticle. It has thick pectinous cell walls and is rich in proteins and

lipids. The embryo is straight and approximately half the length of the seed or a little longer. The cotyledons are about one third of the length of the embryo.

3.2 *Gentiana asclepiadea*

The initiation of the ovule primordium and the integument in *Gentiana asclepiadea* is identical with that in *G. cruciata*. However, the formation of the ovule and the seed is complicated by the development of a seed wing, which already starts during megasporogenesis and gametogenesis. The wing is formed at the raphal, chalazal and anti-raphal sides of the ovule (figs. 14–17). It consists mainly of 2 dermal layers, but at its base some additional periclinal divisions of the subdermal cells take place. At the chalazal side this subdermal activity may be more pronounced (fig. 14). There is a clear difference in shape between the cells of the wing and those of the seed body (photos 9 and 11).

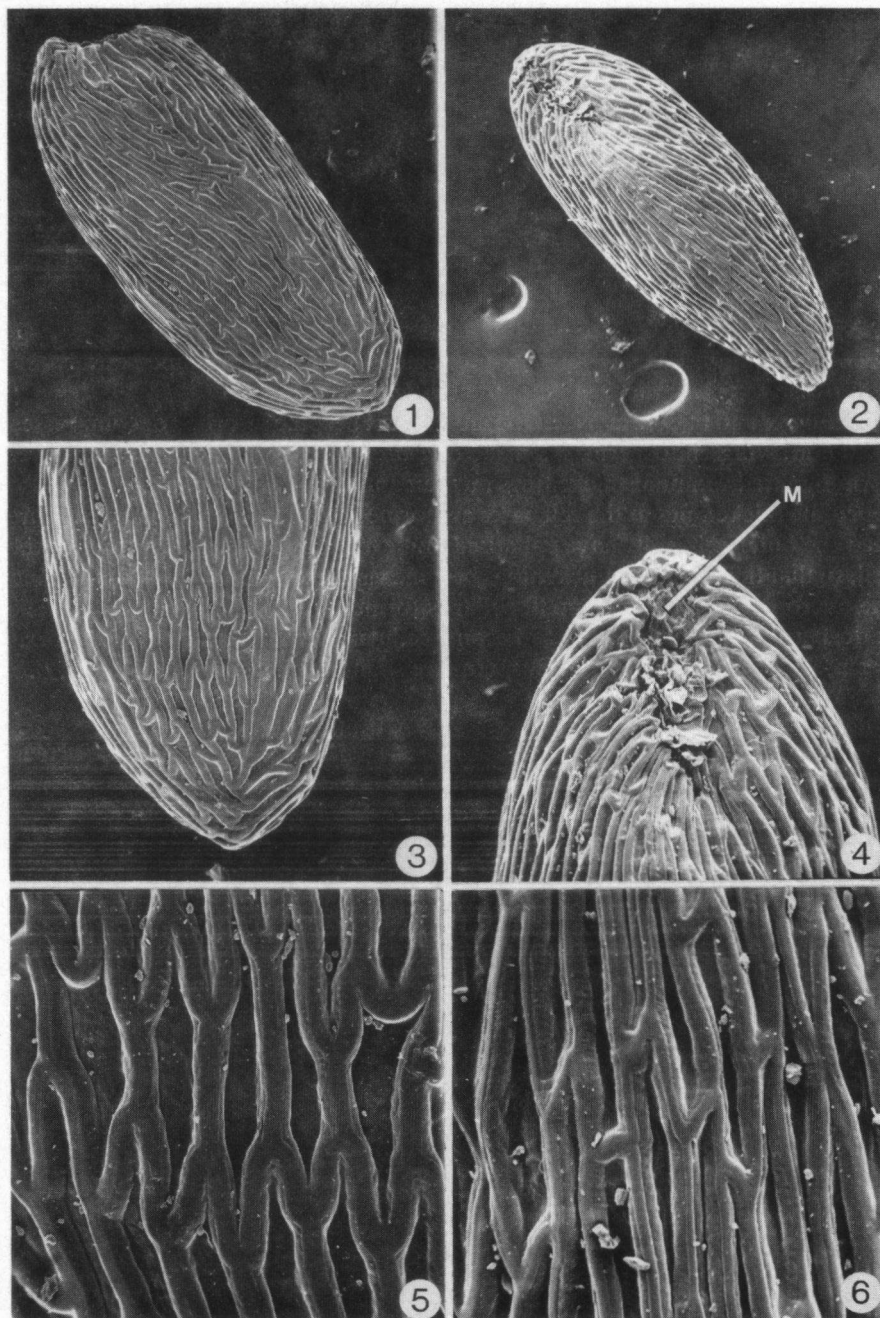
The inner and middle layers of the integument become resorbed or compressed by the growing endosperm. As in *G. cruciata* the cells of the epidermal layer form lignified, annular thickenings on their anticlinal walls, but in addition also reticulate thickenings at the cell bases are formed (figs. 19 and 20). In the mature seed the outer cell walls have collapsed and even may touch the bottom of the cell, so that the reticulate thickenings become visible (photos 10 and 12). Around the micropyle and hilum a small collar is formed (fig. 14 and photogr. 8). The endosperm is initially nuclear to become cellular as soon as the embryogenesis begins. The cell walls of the endosperm are slightly thickened.

4. DISCUSSION

It is clear that the anatomy of the mature seed coat alone is in general of relatively little use in discussions on the phylogenetic interrelationships of the sympetalous families, because almost always only the outer layer of the integument shows any clear differentiation.

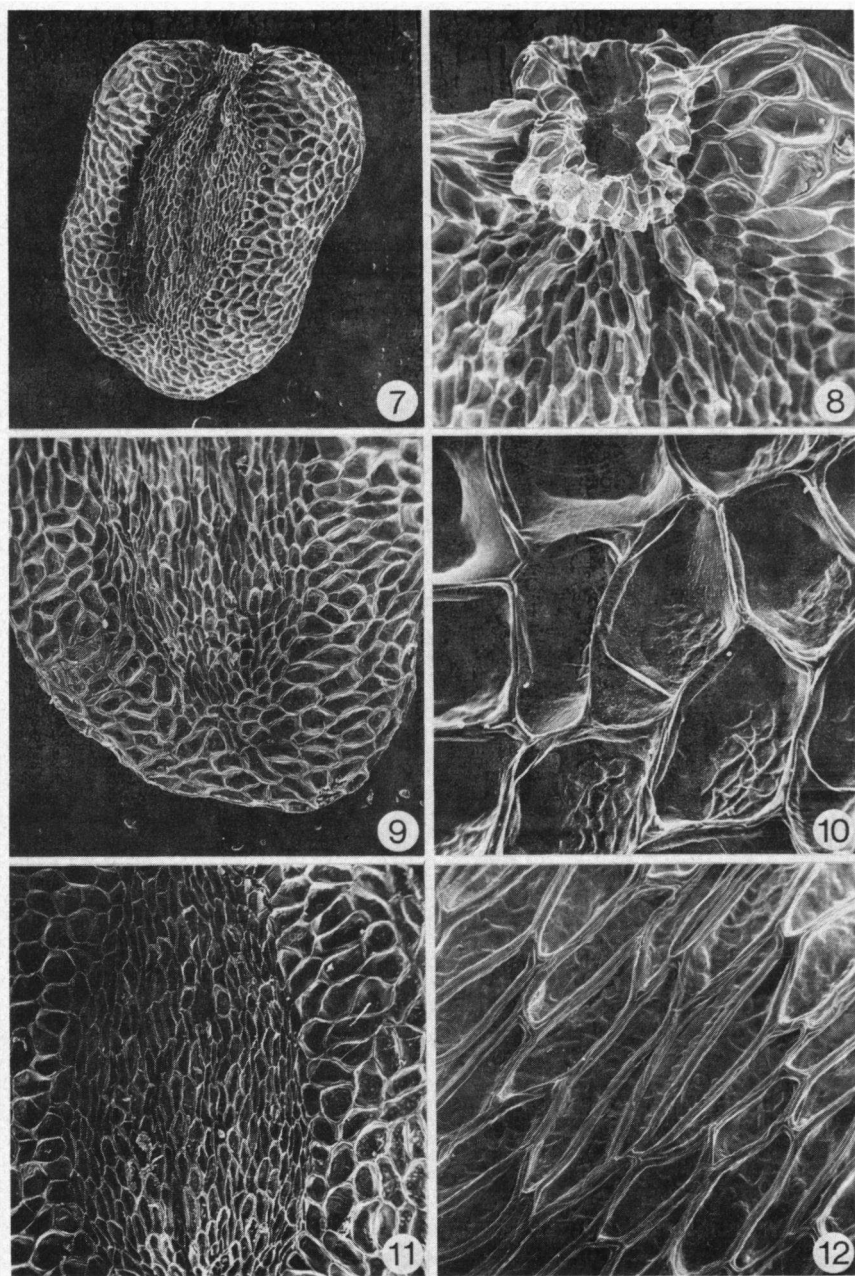
Within the order *Gentianales* the formation of annular thickenings on the anticlinal walls of the outer layer of the seed coat is of rather common occurrence (compare GUÉRIN 1904, CORNER 1976, LÉGER 1913, PAYRAU 1900). It is found in *Gentianaceae* (*Gentiana*), but also in *Loganiaceae* (*Fagraea*), *Apocynaceae* (*Tabernaemontana*, *Strophanthes*, *Ochrosia*, *Voacanga*), *Asclepiadaceae* (*Hoya*) and *Rubiaceae*. This type of thickening is rather rare in dicots and not common in other sympetalous families (it is known to occur in some *Ericaceae*, *Pedaliaceae* and *Lobeliaceae*).

The diversity in ovule and integument initiation within the Sympetalae is greater than can be gleaned from the existing literature. Apart from 2-zonate primordia, there are also 3-zonate primordia which are initiated by periclinal divisions in the third layer of the placenta. It may be expected that this is a character of systematic value generally found above the genus level. In the unitegmic Sympetalae the integument is certainly not always a mere derivative of the epidermis, but may be partly or wholly of subdermal derivation. From studies of ovules showing transitional stages between bi- and unitegmy it is apparent



SEM photographs *Gentiana cruciata* seeds.

Phot. 1. Lateral side 45 \times ; Phot. 2. Raphe side 45 \times ; Phot 3. Chalazal end 100 \times ; Phot. 4. Micropylar end with micropyle (m) and hilum 100 \times ; Phot. 5 and 6. Details of seed surface of lateral and raphe side 225 \times and 160 \times .



SEM photographs *Gentiana asclepiadea* seeds.

Phot. 7. Lateral side 25 × ; Phot. 8. Micropylar and hilar collar 100 × ; Phot. 9. Chalazal side 90 × ;
Phot. 10. Detail of seed wing 250 × ; Phot. 11. Seed body 50 × ; Phot. 12. Detail seed body 250 × .

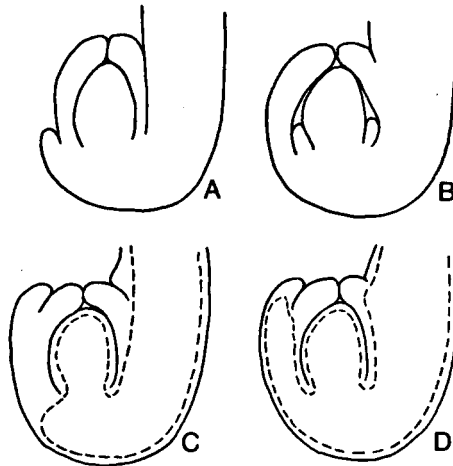


Fig. 21. Intermediate stages in unitegmisation. *A.* reduction of the outer integument, *B.* reduction of the inner integument, *C.* integument fusion, *D.* integumentary shifting.

that there are three possible ways in which the change-over from bitegmy to unitegmy took place (*fig. 21*), *viz.*:

- 1) the reduction of one of the two integuments
- 2) the fusion of integument primordia, which is only possible if the two integuments have the same manner of initiation, *i.e.*, if they are both of dermal origin (subdermally initiated inner integuments are very rare)
- 3) the process of integumentary shifting, which involves a dermal inner and a subdermal outer integument (BOUMAN 1977).

Integumentary shifting has been shown to be operative in *Ranunculaceae* and is likely to take place in the *Rosaceae*. The process of unitegmisation by integumentary shifting is diagrammatically illustrated by four developmental series in *fig. 22*. In the transitional stages B and C the activity of the subdermal cells underlying the dermal initials of the i.i. in conjunction with the activity of the o.i. shifts the i.i. upwards, thus forming a bifid structure. In series D the compound nature of the single integument betrays itself only by the position and the sequence of division of the dermal and subdermal initials, and not any longer by its outline. In the *Gentiana* species studied the integument initiation starts with periclinal divisions in the dermatogen, followed by periclinal divisions in the subdermal layer. This subdermal activity takes place more proximally in relation to the dermal activity. This sequence and position of the dermal and subdermal initials is in accordance with the situation shown in series D of *fig. 22* and renders it plausible that the single integument in the *Gentianaceae* is derived from a dermal inner and a subdermal outer integument by the process of integumentary shifting.

From the pertaining literature and from preliminary personal observations it is evident that not all gentianaceous taxa show the same ovule ontogeny as described above. In *e.g.*, *Exacum* and *Centaurium* the ovule primordium is bi-

zonate and the integument of fully dermal derivation. Ovules without or with only rudiments of integuments are found in the tropical saprophytic genera *Voyria*, *Voyriella*, *Leiphaimos* and *Cotylanthera* (OEHLER 1927). Due to the lack of relevant information, a comparison with the other families of the Gentianales is not possible for the time being.

There is a need for more detailed studies of ovule and integument initiation in the Sympetalae. A better knowledge of these ontogenetic processes will yield more insight into the ontogenetic potentials of the ovule and contribute towards the unravelling of phylogenetic relationships between sympetalous families.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. A. D. J. Meeuse for the critical reading of the manuscript and Ms. N. Devente for technical assistance. The SEM photographs were taken at the Electron Microscopic Laboratory of the University of Amsterdam.

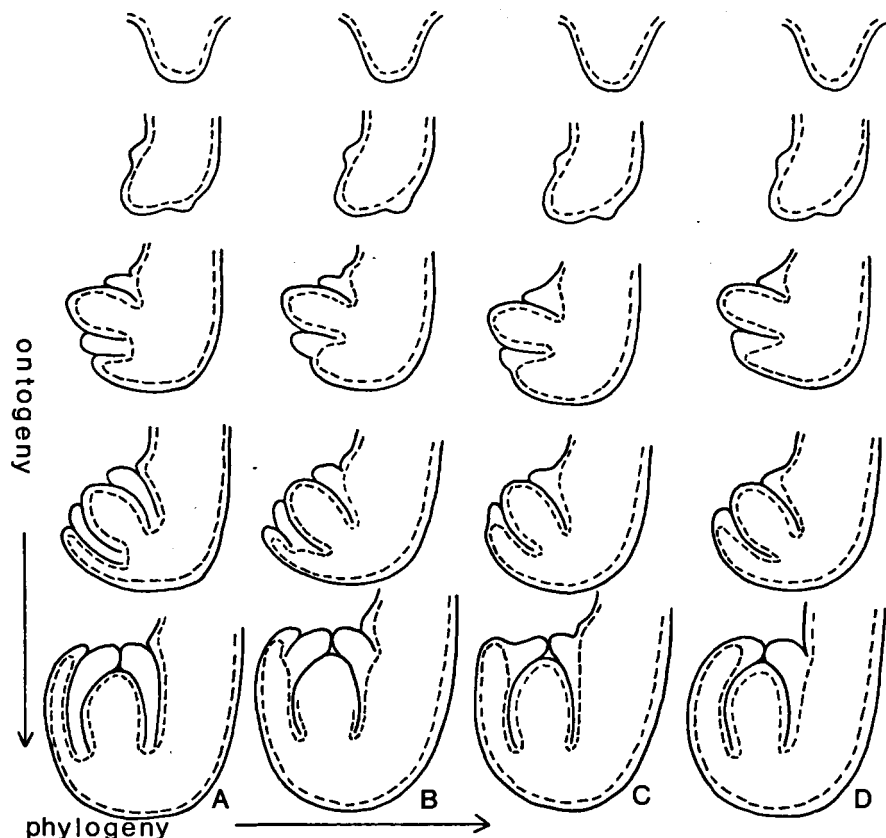


Fig. 22. Developmental series of *A* a bitegmic ovule, *D* an unitegmic ovule, *B* and *C* intermediate stages.

REFERENCES

- BHANDARI, N. N., F. BOUMAN & S. NATESH (1976): Ovule ontogeny and seed coat structure of *Scrophularia himalensis* Royle. *Bot. Jahrb. Syst.* **95**: 535–548.
- BOUMAN, F. (1977): Integumentary shifting – a third way to unitegmy. *Ber. Deutsch. Bot. Ges.* **90**: 15–28.
- CORNER, E. H. H. (1976): *The seeds of dicotyledons*. 2 Vols. London.
- DAVIS, G. L. (1966): *Systematic embryology of the angiosperms*. New York.
- GOVIL, C. M. (1970): Morphological studies in the family Convolvulaceae. 1. Development and structure of the seed coat. *J. Indian Bot. Soc.* **50**: 32–39.
- GUÉRIN, P. (1904): Recherches sur le développement et la structure anatomique du tégument séminal des Gentianacées. *Jour. de Bot.* **18**: 33–52, 83–88.
- KAUR, H. & R. P. SINGH (1970): Structure and development of seeds in three Ipomoea species. *Journ. Indian Bot. Soc.* **49**: 168–174.
- LÉGER, V. (1913): *Recherches sur la structure anatomique du tégument séminal des Apocynacées et des Asclépiadacées*. Thèse, Paris.
- MAHESHWARI DEVI, H. (1962): Embryological studies in the Gentianaceae (Gentianoideae and Menyanthoideae). *Proc. Indian Acad. Sci. Sect. B* **56**: 195–216.
- NETOLITZKY, F. (1926): *Anatomie der Angiospermen-Samen*. Handb. der Pflanzenanat. X. 4. Berlin.
- OEHLER, E. (1927): Entwicklungsgeschichtlich-zytologische Untersuchungen an einigen saprophytischen Gentianaceen. *Planta* **3**: 641–733.
- PAYRAU, V. (1900): *Recherches sur les Strophanthus*. Paris.
- SATINA, S. (1945): Periclinal chimeras in *Datura* in relation to the development and structure of the ovule. *Amer. J. Bot.* **32**: 72–81.
- SCHNARF, K. (1931): *Vergleichende Embryologie der Angiospermen*. Berlin.
- SRIPLING, A. and F. H. SMITH (1960): Anatomy of the seed of *Convolvulus arvensis*. *Amer. J. Bot.* **47**: 386–392.
- WARMING, E. (1878): De l'ovule. *Ann. Sci. Nat. Bot.* (6) **5**: 177–266.