INTEGUMENTARY STUDIES IN THE POLYCARPICAE
V. NIGELLA DAMASCENA L.

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SUMMARY

The ovule primordium of *Nigella damascena* is three-zonate. The nucellus of most ovules is crassinucellate. The inner integument is of dermal origin, whereas the outer integument is initiated subdermally. These ovule characteristics correspond with those of the supposedly most original type of the angiospermous ovule. The ovule can be used as a base of comparison with unitegmic ranunculaceous ovules and with ovules showing a structure intermediate between bi- and unitegmy.

1. INTRODUCTION

The family of the Ranunculaceae is one of the few angiospermous families with bitegmic or unitegmic ovules or with ovules whose structure is intermediate between bi- and unitegmy. Bitegmic ovules are found in e.g. *Adonis, Caltha, Cimicifuga, Nigella, Thalictrum, Trollius*, and *Xanthorrhiza*, fully unitegmic are e.g. *Anemone, Clematis, Myosurus*, and *Ranunculus*. "Transitional" ovules have been reported to occur in species of e.g. *Eranthis, Delphinium*, and *Helleborus*. A comparative ontogenetic study of ovule and integument initiation may yield a deeper understanding in what way(s) and by which processes the evolutionary change-over from bitegmy to unitegmy has taken place in this family, and in the angiosperms in general (BOUMAN & CALIS 1977).

The study of the initiation of ovules and integuments has been neglected in ontogenetic and embryological papers since WARMING (1878). The only available, detailed descriptions of ranunculaceous ovules are of *Aconitum napellus* and *Delphinium elatum* by STRASBURGER (1872).

Studies on the formation and structure of the seed coat are more numerous, although scattered in the embryological, pharmacognostic, and agricultural or horticultural literature. Surveys on the seed coat structure in Ranunculaceae have been given by LONAY (1900, 1907), NETOLITZKY (1926), and CORNER (1976). The seed coat of *Nigella* in particular has been described by GODFRIN (1880), HARZ (1885), HOLFERT (1890), BRANDZA (1891), ATTEMA (1901), LONAY (1900), VAN WISSELINGH (1924), and VIJAYARAGHAVAN & MARWAH (1969b). The papers by Harz, Godfrin, and Holfert describe the structure of the seed coat, but do not deal with the relation of the seed coat layers and the

ovular structures. The descriptions of Brandza and Attema are incorrect as far as the post-fertilization development of the inner integument is concerned. Embryological research on *Nigella* has been carried out by KORDYUM (1957, 1959), LY THI BA (1962), VIJAYRAGHAVAN & MARWAH (1969a, b), and BHANDARI, KISHORI & NATESH (1976).

Nigella damascena has been taken as an object of research, as an example of the bitegmic ranunculaceous ovule and as a basis of comparison with species having ovules with a single or bifid integument.

2. MATERIAL AND METHODS

The material of *Nigella damascena* L. was collected in the Botanic Garden of the University of Amsterdam, fixed in CRAF III, dehydrated in a TBA-series, embedded in paraplast, sectioned at 7 µm, and stained with safranin and astra blue. Mature seeds were embedded in epon and also sectioned by hand for specific staining. SEM photographs were taken with the aid of a Cambridge Stereoscan Mark 2a. Additional information was obtained from slides kindly placed at my disposal by M. R. Vijayaraghavan.

3. OBSERVATIONS

All flowers have normally five carpels, each containing about 20 ovules arranged in two alternating rows and facing each other with their raphes. The ovule primordium has, like those of the other ranunculaceous species described previously, a clearly three-zonate structure. It is initiated by periclinal divisions in the third layer of the virginal placenta. The two outer zones (the dermal and subdermal layers) show only anticlinal divisions in the beginning (*figs. 1* and *2*). Already before the integuments become discernible the ovule primordium starts its anatropous curvature. The inner integument (i.i.) is fully dermal in origin. It is initiated almost simultaneously on all sides of the ovule primordium by periclinal or oblique divisions of two or three neighbouring rings of dermal cells. These initials give rise to a two-layered i.i. The basal part of the i.i. may be three-layered. The i.i. closes over the nucellus to form the endostome by radial elongation and periclinal division of the uppermost cells of the inner layer.

The outer integument (o.i.) is initiated almost simultaneously with the i.i.; however, in contrast to the i.i., by periclinal divisions of the subdermal layer. This periclinal activity starts at the convex side of the ovule primordium under and proximal in relation to the initials of the i.i. (*fig. 4*). The o.i. is 3 to 6 cells in thickness and, except for its one-layered outer and inner epidermis, of subdermal derivation. Only the dermal cells of the apex of the o.i. undergo some periclinal divisions and form a small dermal cap. Also in the mature ovule there is no indication of any development of an o.i. at the raphal side. The o.i. does not overgrow the i.i. and so does not contribute to the formation of the micropyle. The periclinal activity of the subdermal cells underlying the i.i. is
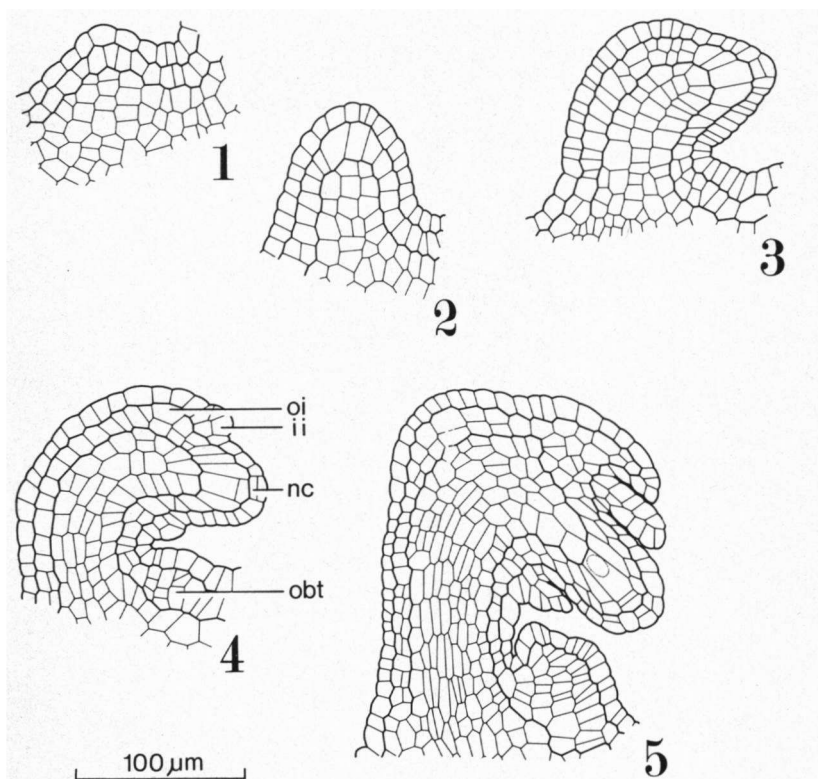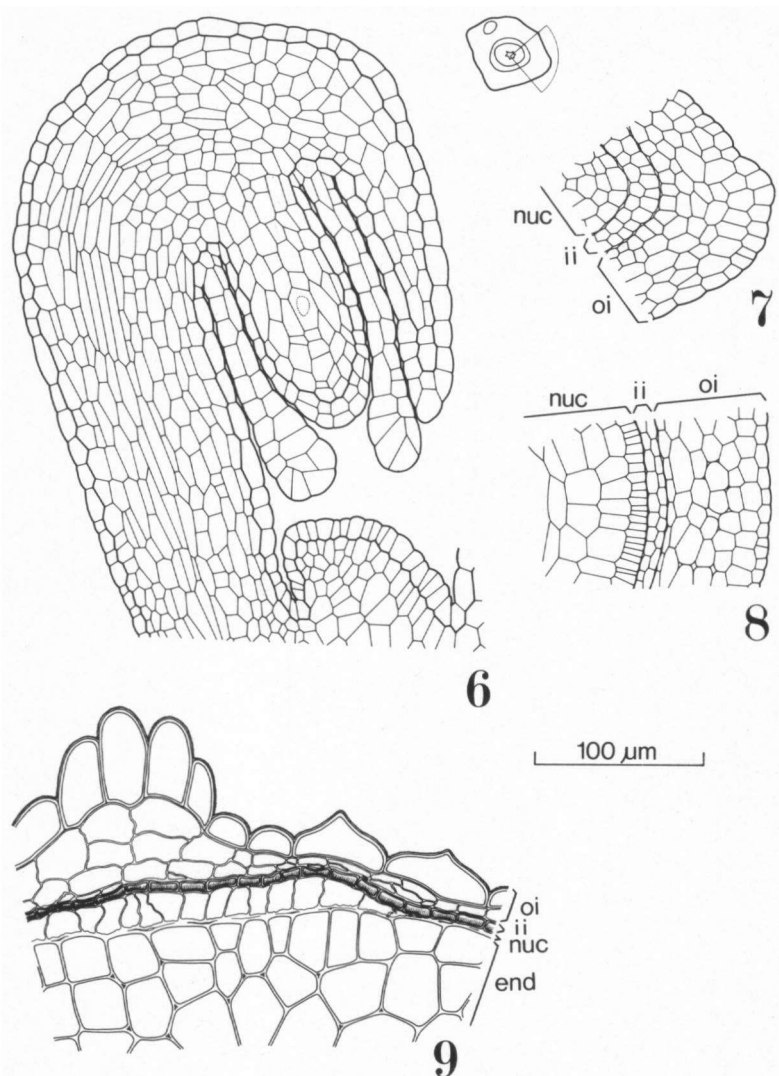
Figs. 1–5. *Nigella damascena.* Longitudinal sections of ovule primordia and young ovules showing ovule initiation, integument initiation and anatropous curvature. oi = outer integument; ii = inner integument; nc = nucellar cap; obt = obturator.

limited and only responsible for a shift of the base-line of the i.i. from a direction in line with the nucellus to a direction perpendicular to it.

In the full-grown ovule the micropyle is plugged by an obturator. This obturator is initiated in a relatively early stage of ovule development (compare *figs. 3* to *6*), is subdermal in origin, and be called placental.

The apical part of the ovule primordium develops into the nucellus. Like the ovule primordium, the nucellus is three-zonate. The outer dermal layer soon undergoes periclinal divisions to form a small nucellar cap of mostly only 2 cells thick (*figs. 3* to *6*). The bulk of the nucellus is formed by the subdermatogen. Its apical cells undergo radial elongation. One of these cells differentiates into an archespore. Usually the archespore divides periclinally to form the megaspore mother cell and the primary parietal cell, thus rendering the ovule crassinucellate. In the mature ovule the parietal tissue is 1 to 4 cells thick. Also the neighbouring cells of the archespore divide periclinally. The central tissue of the nucellus underlying the archespore, megaspore mother cell, embryo sac mother cell or embryo sac, is formed by the third zone. In a

Figs. 6–9. *Nigella damascena*. Fig. 6. Longitudinal section of ovule during megagametogenesis. Figs. 7–9. Cross sections of ovule, and of developing and mature seed respectively. oi = outer integument; ii = inner integument; nuc = nucellus; end = endosperm.

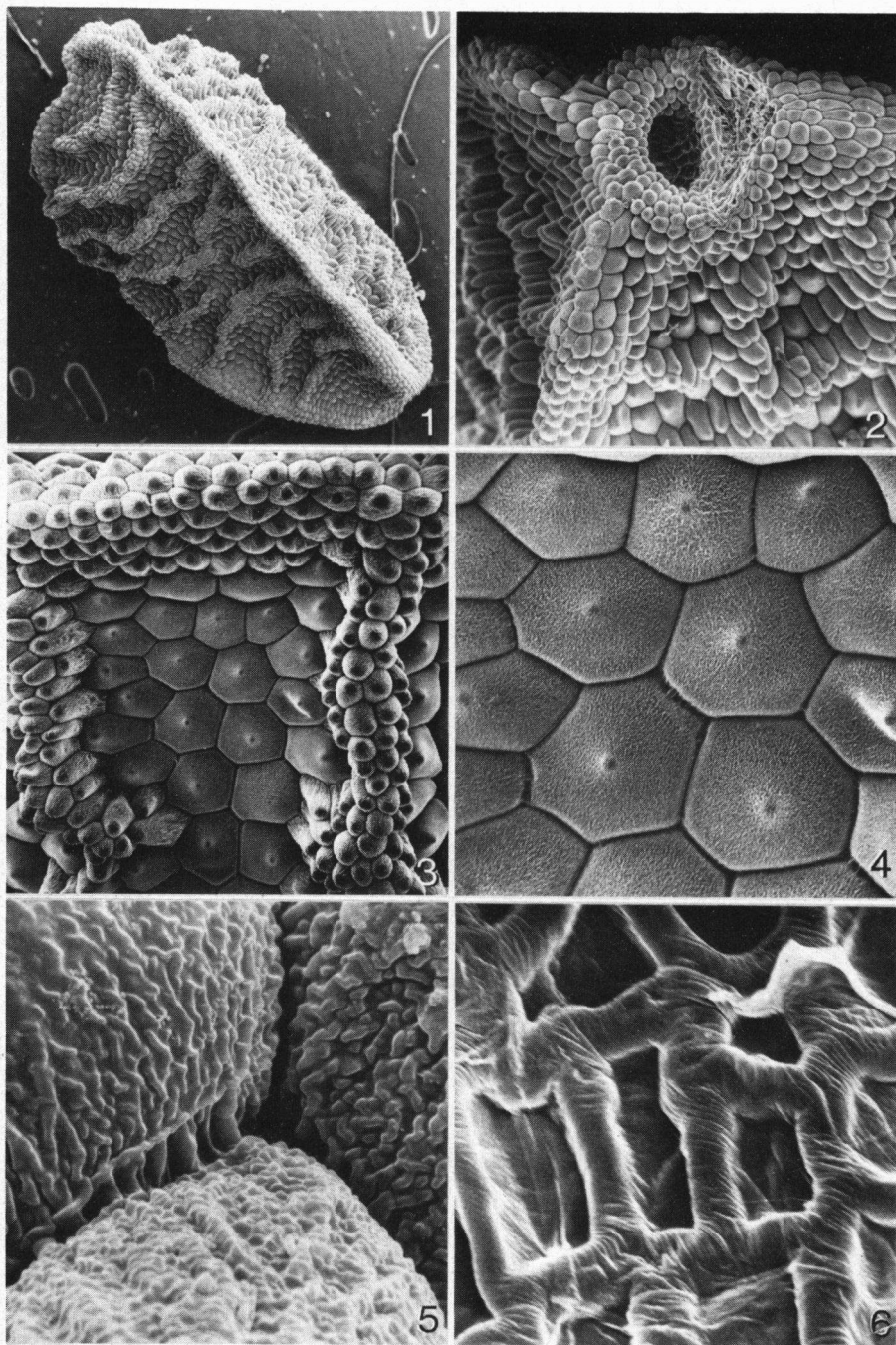SEM photographs. *Nigella damascena*.
Photogr. 1. Mature seed × 25.
Photogr. 2. Hilum and micropyle × 100.
Photogr. 3. and 4. Details of seed coat surface × 100 and × 200.
Photogr. 5. Cuticular texture × 2000.
Photogr. 6. Sectioned seed coat showing detail of the inner layer of the inner integument × 1500.

minority of the ovules the archesporial cell functions directly as the megaspore mother cell. In this case there is no parietal tissue, which makes the ovule tenuinucellate, or, in the terminology of Davis, owing to the presence of a nucellar cap, pseudocrassinucellate. Megasporogenesis takes place at about the moment when the integuments have grown up to the tip of the nucellus. The megaspore tetrads are linear or T-shaped. The chalazal megaspore develops into the embryo sac.

The seed coat is formed by both integuments and by remnants of the nucellus. After fertilization the o.i. becomes 7 to 8-layered, especially by periclinal divisions of the most peripheral cells of the subdermal tissue. This tissue develops small intercellular spaces and assumes a somewhat parenchymatic appearance (*fig. 8*). The outermost cells show a tendency towards radial elongation, the innermost to a more tangential extension. The outer and inner epidermis are clearly discernible. During the later stages of seed development the o.i. is compressed and mostly resorbed from the inside. In the mature seed coat only the outer epidermal layer of the o.i. remains fully intact. Its cell walls become slightly thickened and dark-coloured. The cells stretch in two ways to form erect cells on the ridges and flattened cells in between (*fig. 9*). They may contain tannins.

The i.i. remains 2-layered in the early stages. During the later stages of seed coat development its outer layer becomes compressed and resorbed. The inner layer is very characteristic at the mature stage. The cells are somewhat bone-shaped and contain tannins. The cell walls are thickened, especially the radial ones and slightly lignified. The radial and the inner tangential cell walls develop very fine wall striations (SEM photograph *6*, and *fig. 9*).

The nucellar epidermis forms a very characteristic layer in the developing seeds. Its cells are plasm-rich and divide especially by radial divisions (*fig. 8*), ultimately to become several times longer than broad. The nucellar tissue becomes compressed and partly resorbed by the growing embryo sac. In the mature seed coat the nucellus is present as a compressed layer of cell walls. Only locally do the cells of the epidermis remain discernible as such (*fig. 9*). In the mature seed coat cuticles are present over the outer epidermis of the o.i., and between i.i. and nucellus.

The raphe contains a small collateral bundle with differentiated ring and spiral elements. The bundle ramifies in the chalaza, but does not extend to the o.i. The chalaza remains small and contributes but little to the formation of the seed coat. Its cells on the inner side of the vascular bundles form tannins and may function as a barrier tissue. The endosperm is nuclear and later becomes cellular. It cells show a distinct radial arrangement. The embryo is straight and about as long as half the length of the seed or a bit longer.

In the mature fruit the seeds are closely packed. As a result the seeds become flattened at their mutual tangent planes and at the places where they are in contact with the fruit wall. The mature seeds are 2,5–3 × 1,2–1,8 mm. The seed coat surface shows a more or less reticulate pattern of ridges. The most prominent ridges are formed by the raphe and the tangents of the flattened

planes. The other ridges on the integumentary part of the seed coat are mainly transversely oriented (Photogr. *1* and *2*). The ridges are developed by the activity of both the subdermal and the dermal layers. The subdermal layer of raphe and outer integument shows locally more conspicuous periclinal divisions, whereas the surmounting epidermal cells stretch radially and become papillate (*fig. 9*). The epidermal cells between the ridges expand especially in the tangential direction. These cells remain flat and are penta- or hexagonal in surface view. All the epidermal cells have a nipple-like projection, however. These projections are not so distinct on the cells of the ridges (Photogr. *3* and *4*). The epidermal cells are covered by a characteristically textured cuticle (Photogr. *5*).

4. DISCUSSION

The basic nature of the bitegmic and crassinucellate, angiospermous ovule was generally accepted by the turn of this century. More recently the present author (BOUMAN 1974) postulated that the most primitive type of angiospermous ovule has a 3-zonate primordium, a dermally initiated inner integument and a subdermally initiated and primarily vascularised, outer integument.

The ovule ontogeny of *Nigella damascena* has been investigated as a basis of comparison with that of ranunculaceous taxa having unitegmic ovules or ovules intermediate between bi- and unitegmy. In *Nigella damascena* the ovule primordium is 3-zonate, which implies that the ovule is initiated in the third zone of the placenta. The nucellus is crassinucellate in most ovules. The inner integument is of dermal origin, whereas the outer integument is initiated subdermally. A preliminary survey of some other taxa (*Aconitum, Aquilegia, Consolida, Thalictrum*) shows that the above-mentioned characteristics are probably representative of all or at least of most of the bitegmic ranunculaceous taxa. Vascular bundles are present in the outer integument of *Thalictrum minus* (personal observations) and in *Glaucidium palmatum* (KUMAZAWA 1938). The characteristics of the bitegmic ranunculaceous ovule thus appear to agree to a great extent with those supposed to be characteristic of the more primitive kind of angiospermous ovule. In their ontogeny they link up with ranunculaceous forms showing transitional stages between bi- and unitegmy. The present author (see BOUMAN & CALIS 1977) has found that the bifid integuments of *Eranthis, Delphinium* and *Helleborus* are formed by a complicated ontogenetic process called integumentary shifting. This process involves: 1. a fusion of primordia in the sense that the initials of the inner and outer integument give rise to an at least partly common structure; 2. a shifting of the inner integument in relation to the outer one caused by periclinal divisions of the subdermal cells underlying the dermal initials of the inner integument; 3. an arrested growth of the inner integument. If and to what extent the process of integumentary shifting is still recognizable in the wholly unitegmic ovules of the Anemoneae and Ranunculeae needs further study.

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