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DEVELOPMENT OF OVULE AND TESTA OF GERANIUM PRATENSE L. AND SOME OTHER REPRESENTATIVES OF THE GERANIACEAE

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SUMMARY

The ovules and seeds of the genera *Geranium*, *Pelargonium*, *Erodium*, *Monsonia*, and *Sarcocaulon* are very similar. The ovule primordium of *Geranium* is trizonate. The outer and inner integuments are both of dermal derivation and initially only 2 cells thick, to become multilayered owing to mitotic activity in the inner cell layer. Shortly before fertilisation a development begins which ultimately results in a marked degree of campylotropy of the seeds. The mechanical layers consist of the sclerotised and crystal-containing cells of the inner epidermis of the outer integument, and sclerotised cells of the outer layer of the inner integument which are star-shaped in surface view. Nucellus and endosperm have disappeared almost completely in the mature seed. In several representatives of the family the seed-coat contains stomata.

1. INTRODUCTION

The family Geraniaceae *sensu stricto* (= Geranieae *sensu* Knuth in ENGLER & PRANTL 1931) is very homogeneous and contains only herbaceous to suffruticose representatives.

The first publications dealing with the seed anatomy of the family are those of STRANDMARK (1874), RAUNKIAER (1888), BRANDZA (1891), GUIGNARD (1893), and MARLOTH (1883). Raunkiaer was the first to describe the late campylotropy, the development of the seed-coat, and the presence of stomata in the seed epidermis. Of a later date are the contributions of BILLINGS (1901), ATTEMA (1901), LONAY (1904), VAN WISSELINGH (1922), SCHÜRHOFF (1924), NARAYANA & ARORA (1963), NARAYANA (1970), TOKARSKI (1972), and KUMAR (1976).

Date on ovules and seeds of Geraniaceae were summarised by NETOLITZKY (1926), DAVIS (1966), and CORNER (1976). By consensus of opinion the geraniaceous ovules are anatropous, bitegmic, and crassinucellate. The mechanical layers of the seed-coat are formed by the sclerotised cells of the inner layer of the outer integument and the outer layer of the inner integument.

The present study was undertaken mainly to provide detailed descriptions of the ontogeny of the ovule and integuments.

2. MATERIALS AND METHODS

The material of *Geranium pratense* L., *G. molle* L., *G. robertianum* L., *G. macror-*

rhizum L., *G. phaeum* L., *Erodium cicutarium* L., and *E. manescavi* Coss. was obtained from plants growing in the Hortus Botanicus, University of Amsterdam, that of *Geranium sylvaticum* L. from the Hortus Botanicus, University of Leyden. Seeds of *Monsonia speciosa* L. were kindly supplied by H. J. F. Venter (South Africa); seeds of *Sarcocaulon* spec. div. were received from Dr. R. O. Moffet (South Africa).

Craf or Allen-Bouin mixtures were used as fixatives. Sections were cut by means of standard microtome techniques or by hand. Epon was also used as the embedding medium. The following specific stains were used: phloroglucinol-HCl, sudan IV, ruthenium red, and JKJ. The SEM study was carried out with a Cambridge Stereoscan Mk. 2a of the University of Amsterdam after the specimens had been gold-sputtered for three minutes.

3. RESULTS

The representatives of the genera *Geranium*, *Pelargonium*, *Erodium*, *Monsonia*, and *Sarcocaulon* have a 5-loculed ovary with 2 ovules in each locule. The dry fruit falls apart at maturity into 5 mericarps, leaving a central column. Each fruit locule contains only a single seed as a rule, but in mericarps of *Geranium sylvaticum* two much flattened seeds were repeatedly found in superposition.

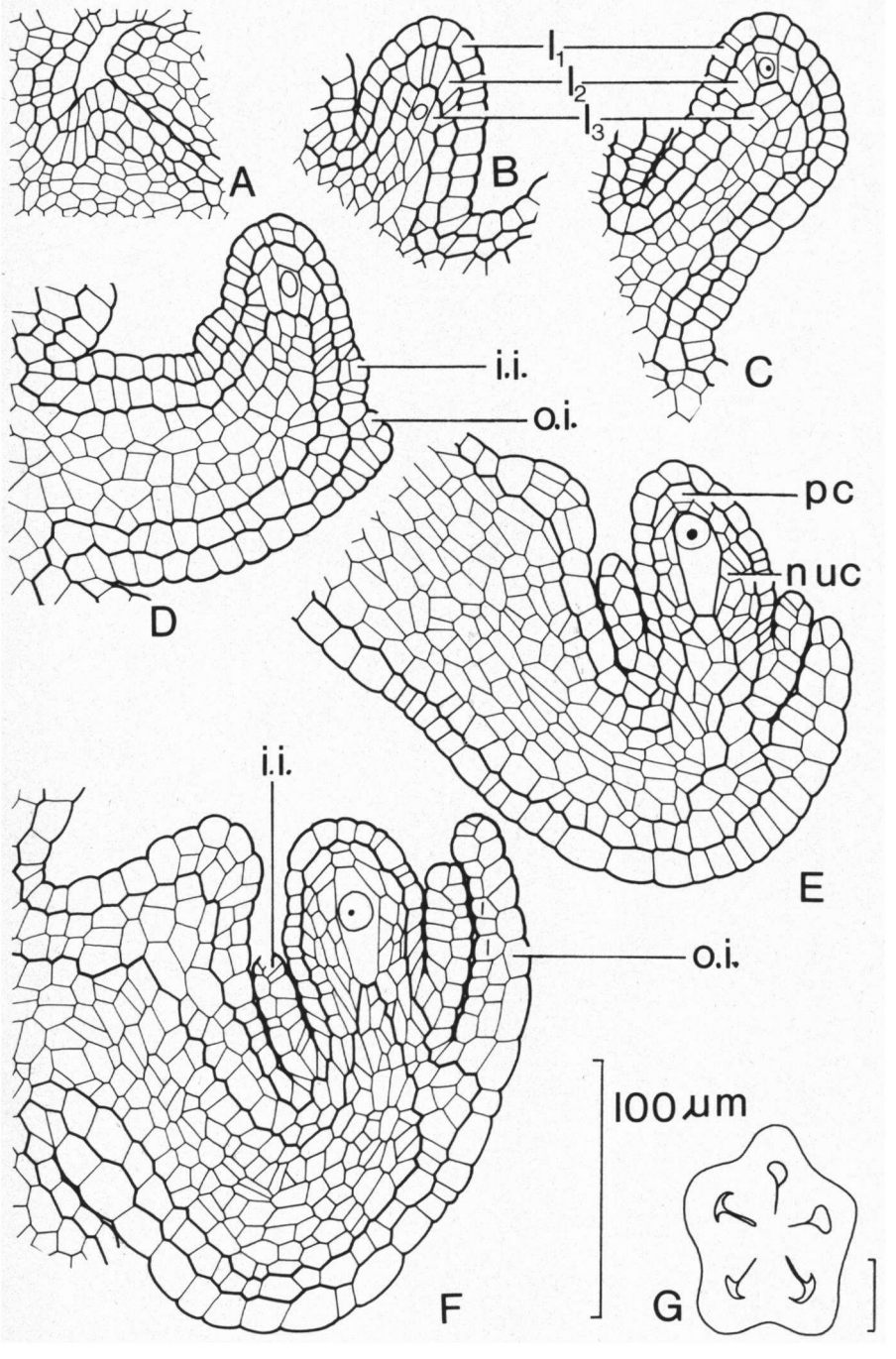
Unless otherwise stated, all data presented refer to *Geranium pratense*.

3.1. Ovule ontogenesis

The ovule primordium of *Geranium pratense* is trizonate. The initiation takes place by periclinal divisions in the third layer (L3) of the placenta, the resulting bulge being covered by the primarily only anticlinally dividing subdermatogen (L2) and dermatogen (L3), see *figs. 1A, B*.

The nucellus is formed by repeated periclinal divisions of the subdermal layer of the primordial apex (*figs. 1C–F*). A subdermal archespore cell divides itself into a primary parietal cell and a megaspore mother cell. A conspicuous parietal tissue 4–6 cells thick is formed and the nucellus becomes rather bulky. Shortly before the ovule is full-grown the epidermis of the nucellus top forms a small cap (*fig. 2B*). In the mature ovule the much enlarged embryo sac (e.s.) has already resorbed all of the distal nucellar tissue (*fig. 2C*). Already before integument initiation has begun the anatropous curvation becomes noticeable.

Fig. 1. Development of ovule of *Geranium pratense*.
A: ovule primordium (detail of *fig. 1, G*).
B, C, D, E and F: l.s. of developing ovules, successive stages.
G: transverse section of ovary with 5 locules.
In all figures and Text: L1, L2 and L3 stand for: dermal layer, subdermal layer and corpus, respectively;
es = embryo sac; em = embryo; cot = cotyledon; end = endosperm; nuc = nucellus; pc = parietal cells; ii = inner integument; oi = outer integument; pvs = provascular strand; ob = obturator; sto = stoma;
all measures indicated correspond with 100 μm.


A
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l_1
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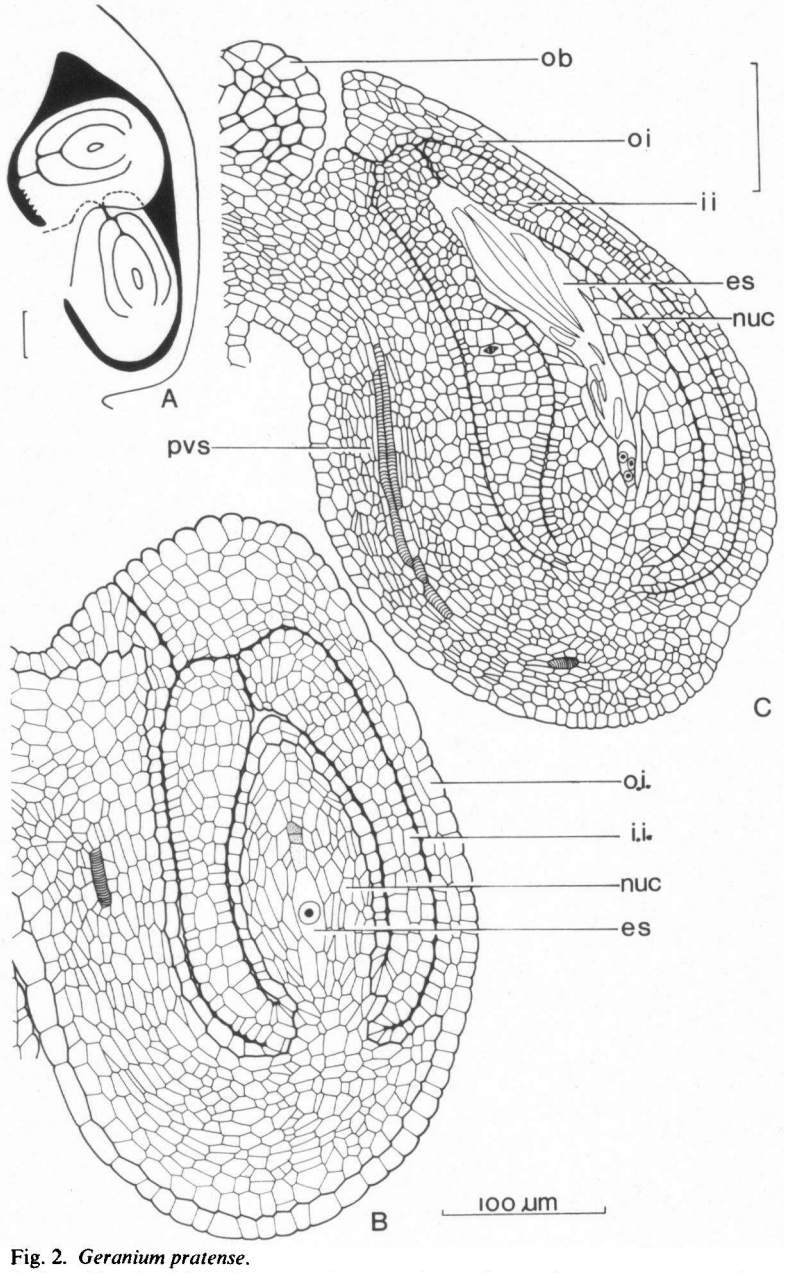

Fig. 2. *Geranium pratense*.
A: l.s. of locule with 2 almost developed ovules; B: l.s. of almost developed ovule: C: l.s. of fully developed ovule.

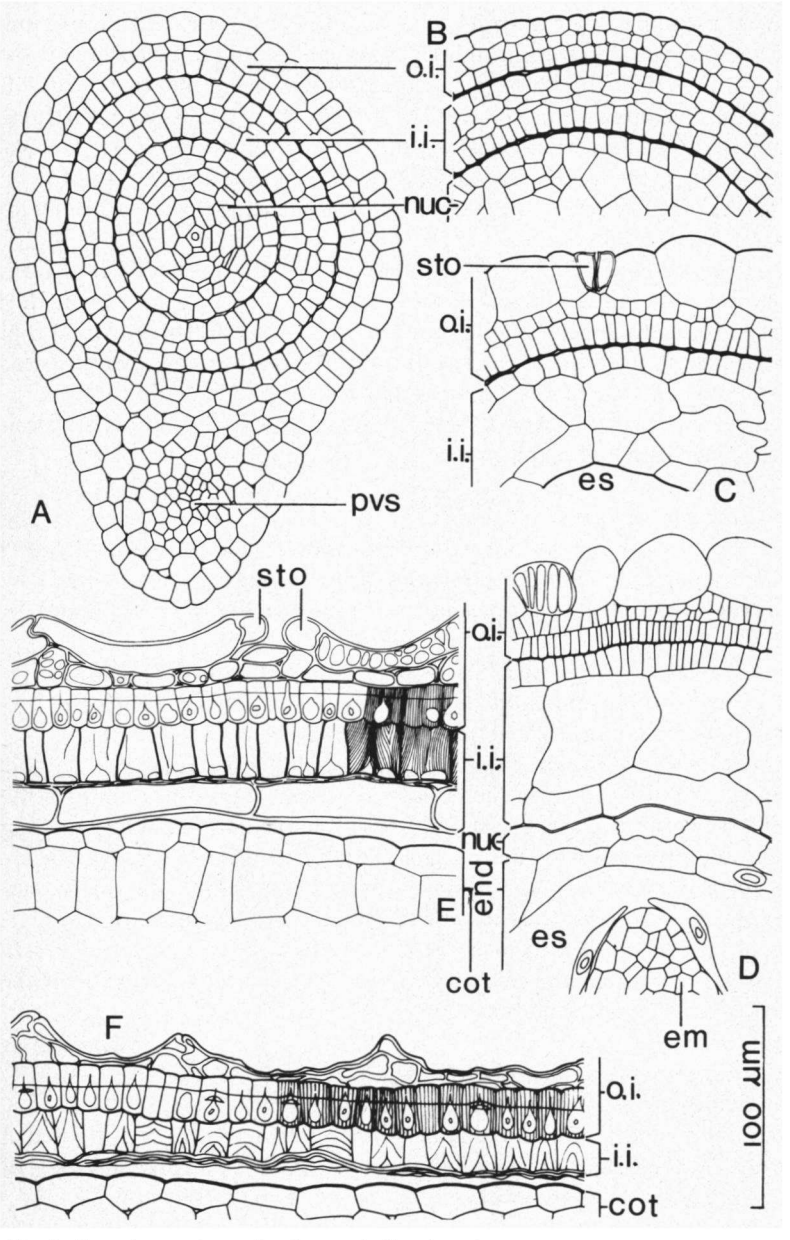

Fig. 3. *Geranium pratense*, development of seed-coat.
A: t.s. of almost developed ovule: B, C, E: t.s. of developing seed-coat: D: l.s. of developing seed-coat; F: t.s. of mature seed-coat; C and E show stomata.
Fig. 3E. corresponds with *fig. 6F.*

The inner integument (i.i.) begins as a complete ring wall resulting from periclinal and oblique wall formation in a band of about three adjacent rows of dermal cells (*fig. 1D*). Owing to the delayed activity of one of the rows of initials the i.i. initially develops as a mainly two-layered structure which becomes three-layered by means of periclinal divisions in the innermost layer (see *fig. 3A*). Shortly before the ovule is fully developed periclinal divisions of the middle layer of the i.i. in the part along the raphe give rise to the campylotropous curving of the ovule (*fig. 2C*).

The outer integument (o.i.) develops simultaneously with the i.i. or a little later, and also by periclinal and oblique cell wall formation in a band of about three rows of dermal cells. In this case the ring wall is first initiated at the convex side of the ovule. As the i.i., the o.i. is originally two-layered but becomes three-layered already in a younger stage of development, likewise by periclinal divisions in the cells of its inner layer. At the stage depicted in *fig. 1F*, the first periclinal divisions of the inner layer have just taken place.

3.2. The fully developed ovule

The full-grown ovule is anatropous with a transition to anacampylotropy, and crassinucellate-bitegmic. The anatropous curvation results in the changing of the place of the raphe in respect of the orientation of the nucellus (*figs. 1C–F* and *2b, C*).

The upper portion of the nucellus is absorbed, but in other taxa such as *Pelargonium tomentosum* and *Erodium manescavi* the distal nucellar tissue stays intact much longer than in, e.g., *Geranium pratense*.

The part of the i.i. facing the raphe has, by periclinal divisions of its middle layer, formed a small protusion which is later responsible for the bending of the nucellus and the embryo sac (e.s.) (*figs. 2C, 4B*). The two peripheral layers of the i.i. and the innermost layer of the o.i. are rich in cytoplasm. The zig-zag micropyle is formed by both integuments. Exo- and endostome are slit to star-shaped in transverse section, the slit(s) being situated in the plane of the funicle. A minute obturator consisting of somewhat mucous cells connects the cells lining the stylar canal and the micropyle (*fig. 2C*). Amyloplasts occur in the apical parts of integuments and nucellus.

3.3. Seed and seed-coat development

3.3.1. Campylotropy

The two obliquely superimposed ovules of *Geranium pratense* are not oriented in the same way. As a rule the upper one has a more transverse position in respect of the longitudinal direction of the ovary compartment than the lower one (*fig. 2A*). Only the upper one develops into a viable seed. According to RAUNKIAER (1888) this must be explained by the failure of the pollen tubes to reach the lower ovule because the funicle of the upper ovule lies over the micropyle of the one below it.

During and after fertilisation a special development takes place which ultimately renders the seed campylotropous, see *fig. 4*. The most important aspects of this process are:

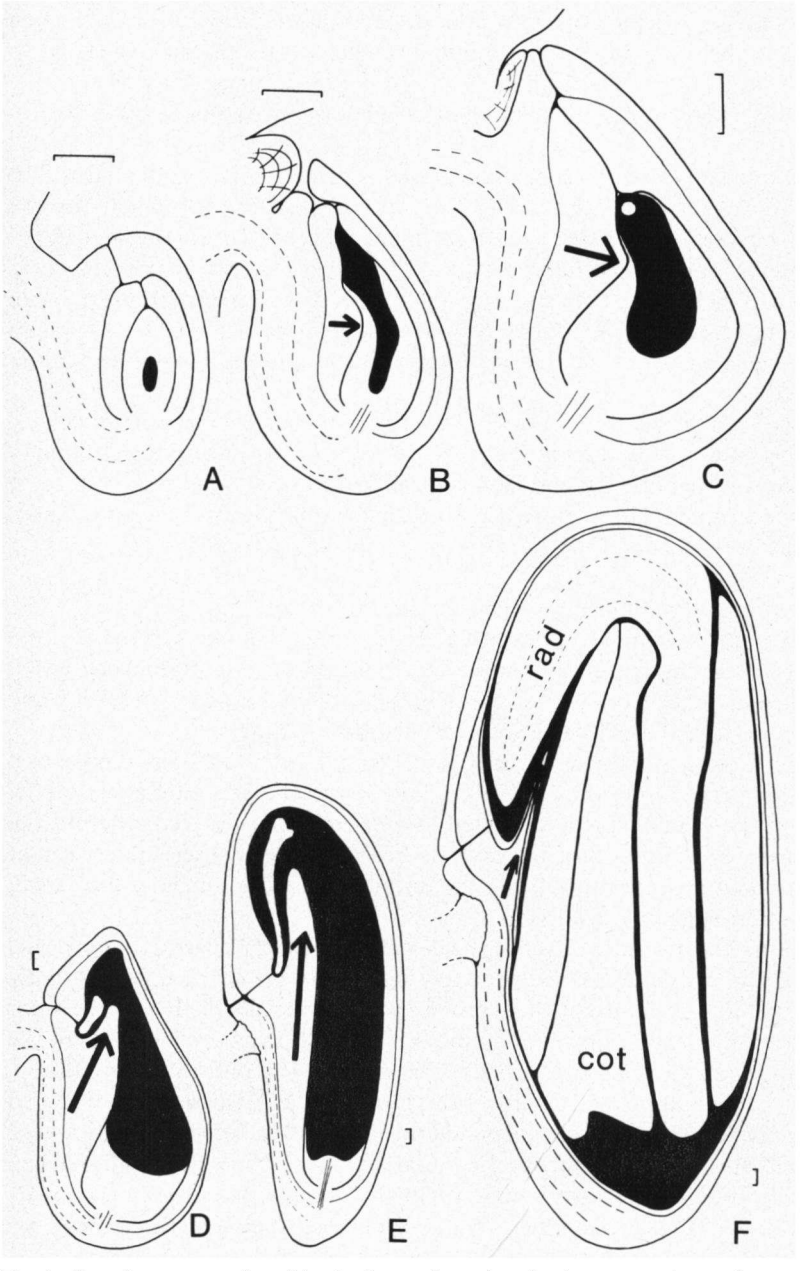

Fig. 4. *Geranium pratense*, l.s. of developing ovules and seeds; the arrow points at the septum of the i.i., responsible for the campylotropy of the seed; *fig. 5.* 4A, B, E correspond with *fig. 5.* 2B, 2C and 3D, respectively.

(1) The occurrence of periclinal divisions in the middle layer of the i.i. on the side of the raphe so that a kind of septum is formed protruding into the embryo sac.
(2) A relatively strong development of especially the anti-raphal side of the seed-coat.

The orientation of the septum thus formed is primarily perpendicular to but later oblique in respect of the raphe. The septum starts to grow chiefly by cell divisions, but later by an extensive stretching of the cells. During the later stages of seed development the chalazal part of the seed extends appreciably in a downward direction so that the originally more transversely oriented ovule develops into a seed oriented lengthwise in the ovarial locule (*fig. 4E*). These developments cause the more lateral, ultimate position of micropyle and hilum in the seed (*fig. 4*).

Sinse the embryo sac is more or less U-shaped, the embryo assumes the same shape. The radicle is situated in the micropylar part of the e.s. and is gradually pushed forward against the cotyledons developing in the other arm of the e.s. Finally, the septum is almost completely crushed flat between the radicle and the cotyledons (*figs. 4F, 6C*).

3.3.2. Seed-coat development

Originally there is a thin layer of nuclear endosperm along the periphery of the e.s. In the lower region of the e.s. no cell wall formation takes place. Where wall formation does take place the endosperm is one-layered except around the young embryo, and there the cell size is also very variable (*fig. 3D*).

The nucellus is gradually resorbed, but all around the e.s. a very thin layer of it persists for a fairly long time (*fig. 3D*), and at the chalazal side a small part remains which later forms a suberised stopper (*figs. 4D, E, 6D*). Before the dermal layer of the nucellus has disappeared a cuticle is discernible without special staining procedures. During the initial stages this cuticle gives a weak reaction when Sudan IV is applied.

The cells of the innermost layer of the i.i. enlarge, stretch tangentially, and later become strongly tanniferous. Immediately before they are crushed flat a radially oriented, pit-like structure of the walls is visible. The cells of the middle layer become very large and poor in content; they become crushed sooner than the cells of the innermost layer (*fig. 3E*). The cells of the outer layer of the i.i. repeatedly divide anticlinally with both lengthwise and transversely oriented walls (*fig. 3C, D*). At the micropylar side this outer layer becomes multilayered and its cells stretch appreciably in the radial direction (*fig. 6C*). At the chalazal end the cells of this layer stretch only somewhat radially, but they do not divide periclinally (*fig. 6D*). During later stages of development wall thickenings are formed especially in the peripheral parts of the cell wall.

The o.i. is, like the i.i., in the young ovule only three cell layers thick, but may locally become pluri-layered by periclinal divisions of cells of the middle layer, e.g., in the micropylar area. The cells of its inner and middle layers are originally very rich in cytoplasm and frequently divide anti-clinally (*fig. 3C, D*). The cells of

its inner layer enlarge and later become, more or less one-sided, strongly sclerotic, whereas those of the middle layer remain more parenchymatic and have hardly thickened walls and numerous intercellular spaces (*figs. 3E, 6F*). The cells of the outermost layer of the o.i. later become very large (*fig. 3E*), somewhat compressed and tanniferous. In these cells, as in all other non-sclerotised testal cells and the cells of the embryo, pit fields can be observed in lateral views of the hardly thickened pectinose walls (*figs. 3D, E*).

Already at an early stage of development of the o.i. stomata differentiate in the outer layer. The guard cells are much smaller than the other dermal cells (*fig. 3C*) but later they enlarge to some extent and the slit becomes wider (*figs. 3E, 6B*). Beneath the stomata the subdermal cells divide periclinally and sometimes a small cavity becomes visible. The accessory stomatal cells are radially arranged, which arrangement is, also according to RAUNKIAER (1888), the same as that of the stomatal complex of the leaves.

3.3.3. The mature seed

In mature seeds of *Geranium pratense* the raphe, the hilum, and the outline of the rootlet are clearly discernible (*fig. 5A*). The length of the radicle varies from species to species: in *Pelargonium tomentosum* (*fig. 5B*) it is nearly as long as the whole seed (and the raphe is very short); this is also the case in *Geranium molle* and in *G. robertianum*, *Erodium cicutarium* (*fig. 5C*), *Monsonia speciosa* and *Sarcocaulon*. The length of the radicle is correlated with the length of the septum of the i.i. and a measure for the degree of campylotropy.

The seed of *Geranium pratense* is dry, of a dull, dark brown colour, and measures 3 × 2 × 2 mm. The surface of the seed-coat is reticulate, which structure is brought about by the prominent radial walls of the outer cell layer. Between these walls a finer pattern can be observed. Both structural features are, essentially, also present in all other species studied (see, e.g., *fig. 6B*). The radial walls responsible for the reticulate appearance of the testa have weakly developed, pectic cell wall thickenings; they become ultimately somewhat pleated and compressed (see *figs. 3E, F*). Another reason why the radial walls protrude is the greater resistance to compression of the underlying cells of the middle layer of the o.i.

In *Erodium cicutarium*, *E. manescavi*, and *Geranium molle* the surface is formed by smaller cells with thinner radial walls, so that the reticulate pattern is obscure (*figs. 5E, 6A*).

In the fully mature seed-coat the structures described above are not so easily discernible as they are in the immediately preceding stage of development.

The finer structure within the meshes of the reticulation is caused by the cells of the middle layer of the o.i. which also have somewhat thickened cell walls and have not been completely compressed.

The seed-coat of *Geranium pratense*, when viewed in cross-section, consists of the following layers:

(1) The outer layer of the o.i. with a thin and only weakly cutinised cuticle.
(2) The much compressed cells of the middle layer of the o.i. – the cell chape is

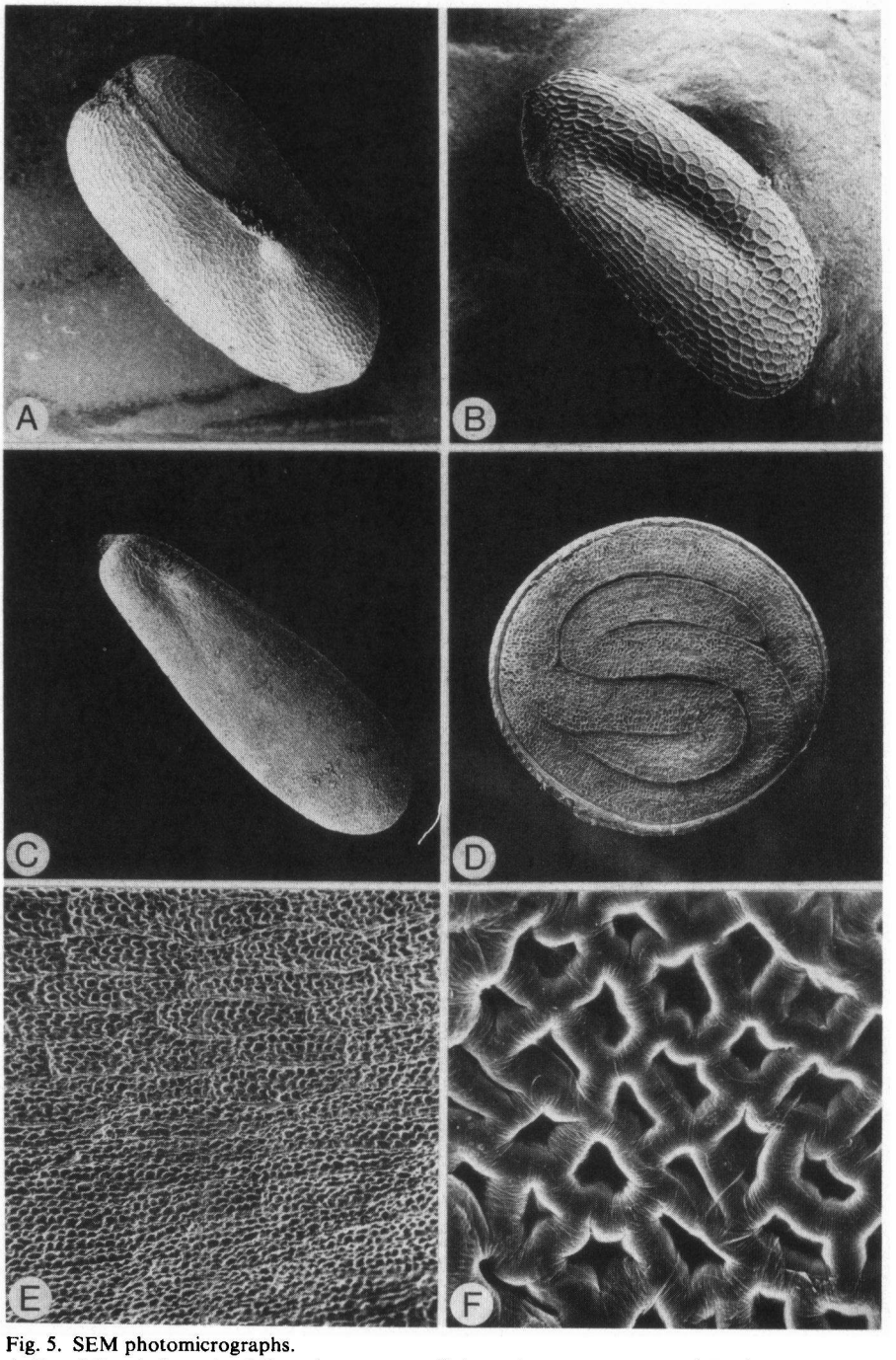

Fig. 5. SEM photomicrographs.
A, B and C: whole seeds of *Geranium pratense*, *Pelargonium tomentosum* and *Erodium cicutarium* (about × 12, × 20, and × 16, respectively);
D: *Geranium sylvaticum*, cross section of seed (× 20);
E: *Erodium manescavi*, seed-coat, outer suface (× 100);
F: *Erodium manescavi*, seed-coat, inner surface (× 800), showing crushed layers overlying the cells of the exotegmen.

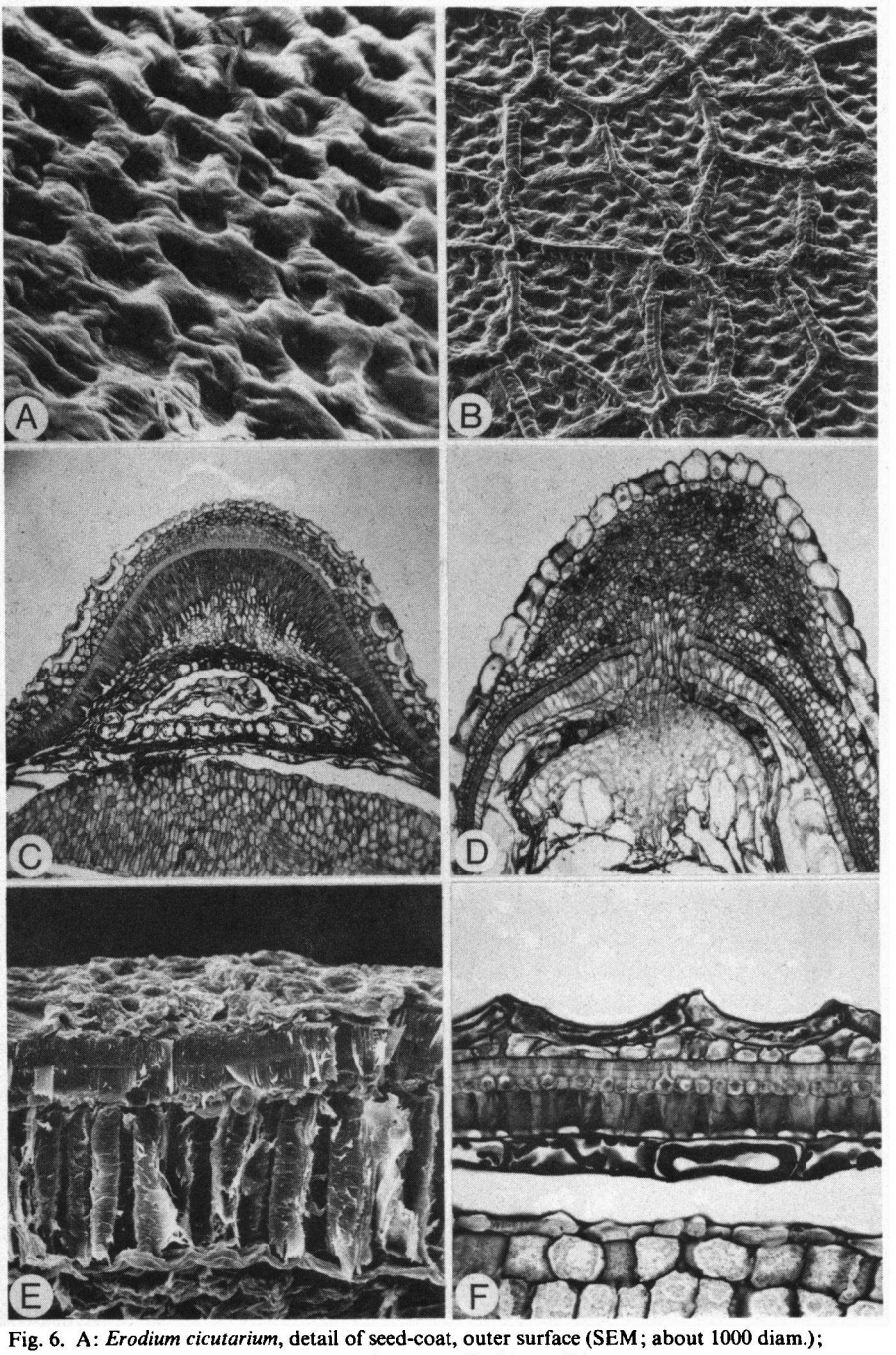

Fig. 6. A: *Erodium cicutarium*, detail of seed-coat, outer surface (SEM; about 1000 diam.);
B: *Geranium sylvaticum*, seed-coat, showing stoma (SEM; × 100);
C: *Geranium pratense*, cross section of micropylar region of almost fully developed seed (light microscope; × 50);
D: *Geranium pratense*, l.s. of chalazal part of developing seed (light microscope; × 75);
E: *Geranium molle*, t.s. of fully developed seed-coat (SEM: × 650);
F: *Geranium pratense*, t.s. of almost fully developed seed-coat (light microscope; × 200).

often still recognisable, as in *Erodium cicutarium* (*fig. 6A*). This middle layer is more than one cell layer thick around the micropyle and at the raphe (*fig. 6C*).

(3) The inner layer of the o.i. consists at this stage of, in suface view, penta- or hexagonal cells with cell wall thickenings which do not show a positive reaction for lignin or pectin; the wall striation is radial (*figs. 3F, 6E*). The lumina of these cells, which contain crystals and tannin, extend as small protrusions beyond the so-called light-line. This light line is also discernible in the SEM micrograph (*fig. 6E*), so that it is not merely an optical effect. In the fully matured seed-coat a number of cells contain a small and possibly tanniniferous body resembling a cap and slightly pushing up the light-line (*fig. 3F*). Near the micropyle the cells are stretched in a radial direction and also contain longer crystals. I.i. and o.i. are tightly connected and their cuticles have disappeared (also according to VAN WISSELINGH 1922); only near the chalazal opening can their cuticles and the nucellar cuticle still be discerned.

(4) The outer layer of the i.i. is developed as an exotegmen and consists of sclerotised cells with in surface view somewhat star-like pleats (TOKARSKI 1972; CORNER 1976; "cells with undulate lobulate facets"). The cell walls contain pectin and lignin. The wall striation is less regular than in the endotestal cells. The wall thickenings are broader (and cuneate) towards the periphery; the basal wall has hardly or not become thickened.

(5) The middle and inner layers of the i.i. are completely compressed the one after the other and both are ultimately resorbed. This process also takes place in the other representatives studied.

(6) The remains of the i.i. layers and those of the nucellus and the endosperm lie against the inner surface of the seed coat and the heavy pressure exerted upon them has partly squeezed them, together with the basal walls, into the lumina of the adjacent exotegmen cells (as shown in *fig. 5F, Erodium manescavi*). This shows that the basal cell wall of the exotegmen is not secondarily thickened. Remnants of the cellular and nuclear endosperm are present around the radicle and near the chalaza, respectively. The inner face of the chalazal opening is covered with a small quantity of suberised tissue. A raphal vascular bundle runs into the chalaza. Around the amphicribral vascular strands tanniferous cells occur.

The embryo is large and in all representatives its cotyledons, when viewed in transverse section, are strongly folded (*fig. 5D*). The embryos of *Geranium pratense*, *Erodium cicutarium*, *Pelargonium tomentosum*, and *Monsonia speciosa* are rich in lipid substances but poor in starch.

4. DISCUSSION

The ovule and seed morphology of the genera *Geranium*, *Pelargonium*, *Erodium*, and *Monsonia* is very similar. The campylotropous seeds of *Sarcocaulon* are not much different (Boesewinkel, unpublished). HOFMEISTER (1858) was probably the first to describe the campylotropy in Geraniaceae. According to PANKOW (1962), the ovule primordium of *Geranium* is dizonate, i.e., a separate subdermal

layer cannot be distinguished. This observation is incorrect.

The integuments of *Geranium pratense* are initially only two and later three or more cell layers thick and are completely of dermal derivation. Only cells of the middle layer of the i.i. divide locally to an appreciable extent, which results in campylotropy.

In *Geranium pratense* cellular endosperm is only formed in the uppermost part of the e.s.; this has already been reported for *Geranium* and also for *Erodium* and *Pelargonium* by GUIGNARD (1893). According to NARAYANA & ARORA (1963) the endosperm of *Monsonia senegalensis* ultimately becomes completely cellular.

In all taxa studied, with the exception of two species of *Pelargonium*, stomata are present in the seed-coat. In view of the fact that the seed-coat is closely adpressed to the ovarial wall these stomata are unlikely to play a part in a process of gas exchange. According to NETOLITZKY (1926) the stomata play a part during the germination process for the intake of water; especially the seeds of species living in arid zones (*Monsonia*, *Sarcocaulon*) must be able to absorb water at a fast rate during a brief period of rain.

The more "typical" families of the Geraniales (Geraniaceae, Linaceae, Oxalidaceae, and Erythroxylaceae) are characterised by an exotegmen sensu Corner. The Geraniaceae are rather singular in that the seed has both an exotegmen and an endotesta. The anatomy and development of the ovules and seeds of the doubtful geraniaceous genera *Biebersteinia*, *Viviania*, *Dirachma*, and *Wendtia* are not or very incompletely known. According to RAUNKIAER (1888) and CORNER (1976) the seed of *Biebersteinia* is not markedly campylotropous, but there is both an exotegmen and an endotesta; according to Corner the complicated morphology of the exotegmic cells of this woody taxon points to an original condition as compared to *Geranium*. Sections of a seed of *Dirachma* studied by the present author (Boesewinkel, unpublished) show the presence of only an exotesta.

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