DEVELOPMENT OF OVULE AND TESTA IN RUTACEAE
I: RUTA, ZANTHOXYLUM, AND SKIMMIA

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SUMMARY

The ovule primordia of *Ruta*, *Zanthoxylum*, and *Skimmia* are trizonate. The initiation and further development of the ovule in the three genera is rather similar, but in *Ruta* and *Zanthoxylum* the outer integument is of dermal origin whereas in *Skimmia* it is partly also of subdermal derivation. The mature seed coats vary appreciably in structure, which may be explained by the different dispersal mechanisms of the fruits or seeds.

1. INTRODUCTION

At the moment there is a renewed interest in the systematics of the Rutales, chiefly owing to chemodiagnostic studies (FISH & WATERMAN 1973, WATERMAN 1975). The order is now more or less directly derived from the Magnoliidae (HEGNAUER 1971, MEEUSE 1970) rather than indirectly (e.g., through Saxifragales: TAKHTAJAN 1973).

Within the key family of the Rutaceae capsules and various (other) types of dehiscent dry fruits, next to samara-like, and baccate ones occur. The formation of the endocarp, usually out of the inner dermal layer of the ovary wall, is the common binding characteristic of the representatives of the family (HARTL 1958). As a result of the varied fruit morphology the number of different kinds of seeds is also large. The ovules are, with few exceptions, bitegmic, crassinucellate, and anatropous ("suspended") or hemi-anatropous with a tendency towards campylotropy. SCHLOTTERBECK (1896: *Ruta graveolens*) and BIERMAN (1896: *Citrus vulgaris*) were the first to study the ovule and the testa of representatives of the family. GALLET (1913) studied the formation of the testa in 28 rutaceous taxa. In his well-known text-book, NETOLITZKY (1926) gave an excellent survey of all data relevant to seed coat development and structure available at that time. In his more recent, comprehensive treatise of dicotyledonous seeds CORNER (1976) treats the ovular and seed morphology as the principal topics. In the present study the ontogenetic development of ovule and testa is given more emphasis. For the classification of the family the system of ENGLER & PRANTL (1931) has been adopted, also followed by Melchior in the revision of the "Syllabus" of ENGLER (1964).

2. MATERIAL AND METHODS

The material of *Ruta graveolens* L., *Skimmia japonica* Thunb., and *Zantho-xylum simulans* Hance was collected in the Hortus Botanicus, Amsterdam (from cultivated specimens). Fixation took place in CRAF mixtures (see SASS 1958: pg. 18). Microtome sections were made following standard procedure and stained with safranin and astra blue. Mature seeds were sectioned embedded in epon. In addition hand sections were made, particularly of mature seeds. SEM photomicrographs were taken with the aid of a Cambridge Stereoscan Mark 2a. The following more specific stains and reagents were used:
(1) phloroglucinol and aniline sulphate for the localisation of lignified cell walls,
(2) Soudan IV for fatty substances,
(3) Ruthenium oxychloride for pectic substances, and
(4) Iodine in potassium iodide solution for starch.

3. RESULTS

A. *Ruta graveolens*
Ruta graveolens (subfamily Rutoideae, tribe Ruteae) has a 4-loculed ovary with per locule 8 ovules in different spatial arrangement and with different orientation of which only some develop into viable seeds (SCHLOTTERBECK 1896). The fruit is a capsule.

Ovular ontogeny
The ovular primordium exhibits a trizonate build-up (see *fig. 1a, b*). The two tunica layers, the dermatogen (l_1) and subdermatogen (l_2), respectively, enclose the corpus (l_3). After the initiation of the ovule by periclinal cell divisons in the third cell layer, both tunica layers initially only divide anticlinally. Subsequently the dermatogen starts to grow also by periclinal cell divisions in a later stage of development. The formation of the sporogenous cell and the parietal ones follows soon thereafter (see *fig. 1, b, c*). The parietal and other cells surrounding the embryo sac divide repeatedly to form a very massive ("prominent") nucellus. The cells around the ES mother cell are more or less clearly arranged in radial rows (*fig. 1 d* and *e*). Shortly before the ovule is full-grown a small cap is formed by cell divisions in the nucellar epidermis. Also below the zones of initiation of the two integuments periclinal divisions take place in the subdermatogen leading to integument initiation.

   The primordium of the inner integument (i.i.) takes the shape of a complete ring wall formed by periclinal cell divisions in two or three adjacent cell rows (seen as 2 or 3 cells in l.s.: see *fig. 1 b*). The i.i. is initially two cell layers thick, presumably because the mitotic activity in one of the dermal initials lags behind (*fig. 1 d*). Already before the ovule has matured the i.i. becomes three-layered by periclinal divisions of the cells of the inner layer (*fig. 1 e, f*). No
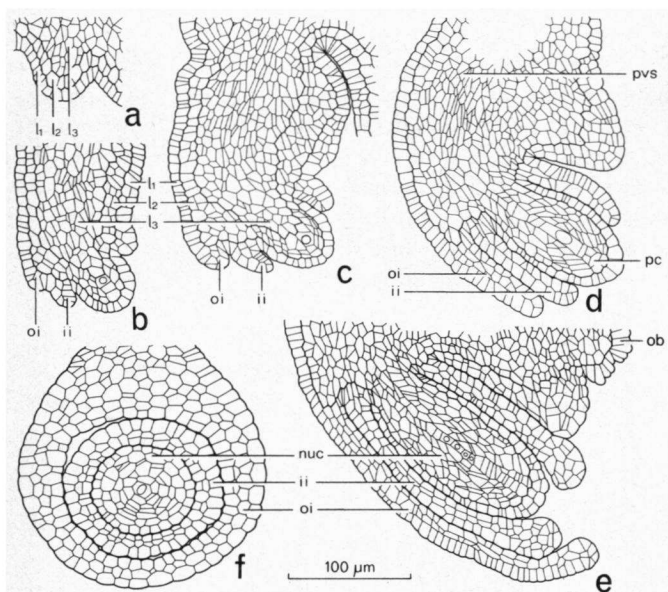
Fig. 1. Ovule development in *Ruta graveolens*. a, b, c, d, and e: successive stages, shown in l.s.; f: stage corresponding with e, in tr. s. In all figures: l₁, l₂, and l₃ = dermal layer, subdermal layer and corpus, respectively; i.i. = inner, o.i. = outer integument; nuc = nucellus; pc = parietal cells, pvs = procambial (future vascular) strand; end = endosperm; ob = obturator.

apical initial could be discerned and the development seems to be mostly homogeneous.

The outer integument (o.i.) originates at the same time as the i.i. or somewhat later, also as the result of periclinal divisions of about 3 rows of dermal cells. For the anatropous curvature of the ovule the delayed initiation of the o.i. at the concave side is characteristic; initially the ring wall formed is incomplete. The o.i. primarily develops as a three-layered outgrowth (*fig. 1 d, e, f*). There are no indications of an apical growth either. By the time the ovule matures the inner epidermal layer has started dividing periclinally. The first periclinal division of this inner layer is shown in *fig. 2 a*. This mitotic activity continues so that the o.i. ultimately becomes 6 to 7 cell layers thick (*fig. 2 b*).

The obturator
The dermal cells lying below the o.i. at the concave side of the ovule gradually stretch themselves in a radial direction. The first clear indication of this cell elongation is already noticeable during megasporogenesis (see *fig. 1 e*). The obturator is, therefore, of funicular derivation. At the time of fertilisation these stretched cells have assumed the shape of unicellular hairs which start to grow in the general direction of the micropyle. After the fertilisation process has been completed these hairs soon disappear.

The full-grown ovule
The anatropous curvature of the ovule is about 90° (*fig. 5 a*). During and after fertilisation the ovule tends to become more campylotropous. The funicle is short but very thick. Starch grains occur throughout the ovular tissue but are especially conspicuous in the apical portions of the nucellus, the o.i., and the i.i. The micropyle is formed by both integuments. The o.i. protudes beyond the i.i.; the endostome and exostome both form a slit in the median plane. According to SCHLOTTERBECK (1896) the exostome shifts its position in the direction of the funicle in respect of the endostome.

Seed and testa development
In the post-fertilisation stage of development the nucellus is gradually re-sorbed with the exception of a group of cells at the chalazal side which soon
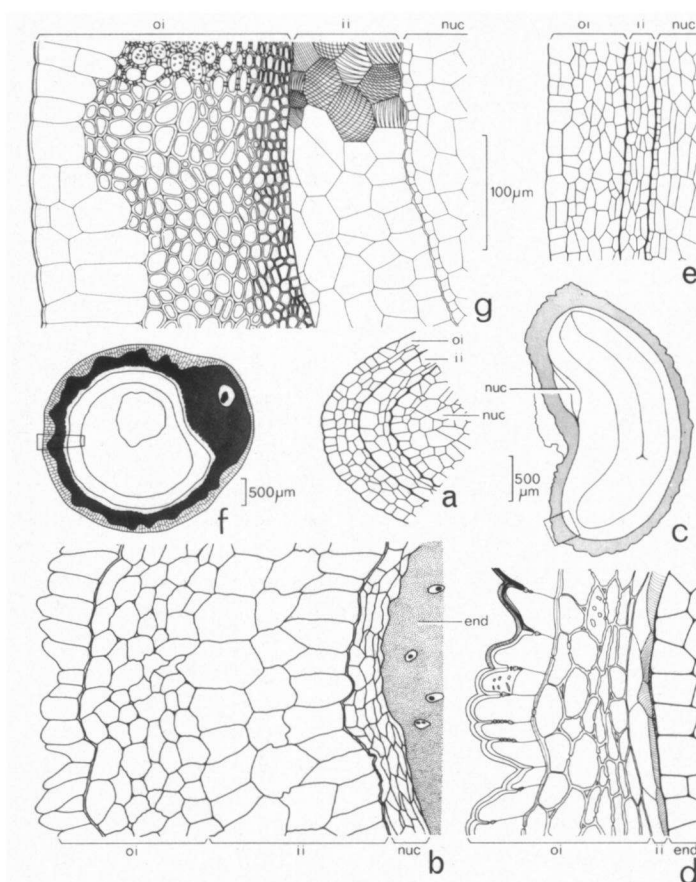
Fig. 2. a, b, c, d: *Ruta graveolens*. a: tr. s. of a part of full-grown ovule; b: tr. s. of developing testa, stage of nuclear endosperm; c: l.s. of mature seed; d: mature testa, tr. s.; e, f, g: *Zanthoxylum simulans*. e: tr. s. of developing testa f: tr. s. of young seed; g: tr. s. of mature testa.

become rich in cytoplasm and more or less assume the character of a hypostase. In the mature seed (*fig. 2 c*) this remaining part of the nucellus lies closely adpressed against the endosperm. The cells of the i.i. are much enlarged and parenchymatic during the phase during which the endosperm is still nuclear (*fig. 2 b*). The cells of the outer epidermal layer have already commenced to elongate in the radial direction.

The mature seed
In the concluding phases of testa development the radial elongation of the epidermal cells of the o.i. continues, but not all cells elongate to the same extent (*fig. 2 d*), groups of more conspicuously stretched cells becoming separated by flatter strips of cells (*fig. 7* and *8*). The ultimate aspect of the seed coat thus becomes somewhat verrucose. On the thickened, multilayered outer walls of the erstwhile o.i. epidermis a layer with radial structure is formed, which coating is referred to by the name of "subgelatinous pellicle" by CORNER (1976, vol. I, pg. 236); see *fig. 2 d*. On *fig. 9* this "pellicle" has become detached from two of the cells. On the walls where the inner structure can be seen because parts of the pellicle layer broke off a radial texture consisting of what seem to be radially oriented tubules becomes visible. The outer surface of the pellicle appears somewhat spongiose owing to the dull black colour of the dry, mature seed. Where the pellicle has disappeared the cell wall proper can be seen, where the o.i. has become torn small pits (or ectodesmata?) can be observed; this photograph must be compared with *fig. 2 d*. Of the i.i. remain only vestiges of the innermost two and much compressed cell layers. The innermost layer exhibits a striation caused by cell wall thickenings (*fig. 2 d*). Here and there remnants of the nucellus and of the nucellar cuticle can be observed, mainly towards the chalaza. In the completely matured and hardened testa only the innermost, striate layer of the i.i. can be discerned as a rule. Also the cells of the o.i. have become more strongly compressed. The chalaza is rather small. The vascular strand inside the funicle is amphicribral (*fig. 10*). As stated before, the dried, mature seeds are a dull black, but they become pitch black when moistened presumably by water imbibition of the "pellicle". By the time the megasporogenesis has been completed the outer two layers of the o.i. and the funicle already contain the brownish, tannin-like products later responsible for the dark colour of the seed. The dimensions of the seed at maturity are about $1 \times 1 \times 2.5$ mm.

The testa is not lignified; the i.i., the nucellar cell walls, and embryo contain pectic substances. Endosperm and embryo are both rich in fatty substances but poor in starch. The nucellar cuticle in particular contains an appreciable amount of fatty or waxy products.

B. *Zanthoxylum simulans*
Zanthoxylum belongs to the subfamily Rutoideae, tribe Zanthoxyleae. In each locule of the ovary there are two ovules in juxtaposition. The fruit is capsular.

Ovular ontogeny

The initiation and development of the ovule are largely similar to those in *Ruta graveolens* (see *figs. 3 a, b, c, d*). As is also the case in *Ruta*, the curvation of the ovule is about 90° (*fig. 5 c*), but there is no tendency towards campylotropy. The ovule primordium is trizonate. Both integuments are of dermal derivation and initially of equal length and three cell layers thick (*fig. 3 c*). The growth in thickness of the integuments takes place by means of periclinal cell divisions of all cells in the o.i. but only of the middle layer in the i.i. (*fig. 3 d*). An obturator is present, but it is very difficult to ascertain whether it is of placental or of funicular origin. In the stage shown in *fig. 3 d* the two or three outermost cell layers of the o.i. and the placental region already begin to accumulate tannic material. By the time the ovule has matured the o.i. protrudes much beyond the i.i. (which does not or hardly extend beyond the apex of the nucellus and is inhibited in its development or even somewhat reduced). The micropyle is formed by the o.i. The exostome is slitlike and extends in the median ovular plane.

Seed and testa development

Already at an early stage of development the outermost cell layer of the o.i. starts to exhibit a singular and very characteristic feature, some cells becoming much more elongate than the other ones and the most strongly elongated ones sometimes dividing once by a periclinal wall (*fig. 2 e*). During later stages of maturation such stretched cells may divide again or even twice (*fig. 2 g*). The later thickening of the o.i. is mostly brought about by periclinical divisions. The i.i. never becomes more than 4-layered. In the full-grown seed coat the
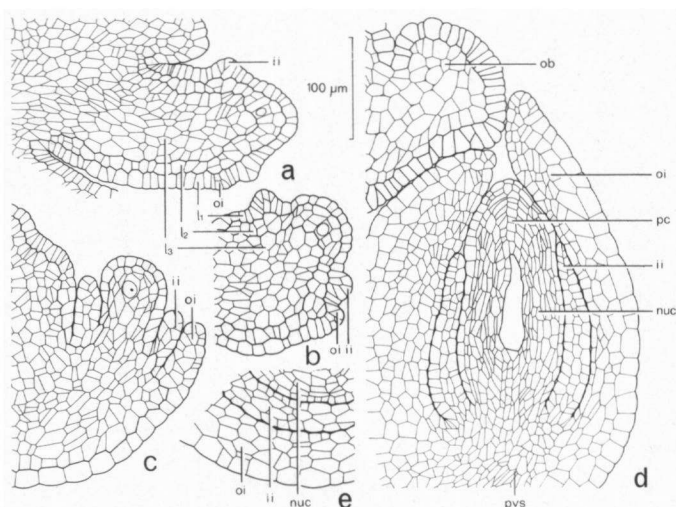

Fig. 3. *Zanthoxylum simulans*. a, b, c, d: l. sns. of developing ovules; e: tr. s. of ovule shortly before its maturation.

cells of o.i. and i.i. are much enlarged. The cells of the o.i. are sclerotised and more or less isodiametric with the exception of the derivative cells of the outermost cell layer which remain thin-walled. The cell walls contains simple pits. The cell walls of the i.i. have striate thickenings and appear to be streaked under the microscope. *Fig. 13* reveals that the striations may be somewhat reticulate. The dermal nucellar cells are stretched in the direction of the longitudinal seed axis and appear as small cells in transverse section. A conspicuous nucellar cuticle is present overlying the remnants of the nucellar tissue.

In the fully mature seed coat no cells have become indistinguishable by resorption. The thin walls of the outer cells of the o.i. become easily damaged. The cells of the i.i. are somewhat compressed. The chalaza is rather small. The funicle trace is amphicribral.

The seed is about $4 \times 4 \times 6$ mm when mature and a shiny black in colour because most of the cells derived from the o.i. contain tannin-like substances (see *fig. 11*). The testa is not lignified. The embryo, the cotyledons, the endosperm, and the cuticle of the o.i., but particularly the nucellar cuticle are rich in lipids. The o.i. is rich in pectic substances. Starch grains occur in the o.i. but particularly around the amphicribral vascular strand of the funicle.

C. *Skimmia japonica*

Skimmia belongs to the subfamily Toddalioideae. *S. japonica* has a 4-pyrenate drupe, and each locule is one-seeded.

Ovular ontogeny

The development of the ovule agrees rather closely with that of the two above-mentioned genera. However, the o.i. is partly of subdermal derivation and already fairly thick (about 4-layered) soon after its initiation: see *fig. 4, c, d, e*. The border-line between the dermal and the subdermal part is later no longer discernible, so that their relative contribution towards the mature testa is difficult to ascertain (*figs. 14* and *15*). The longitudinal growth of the o.i. is mostly accounted for by anticlinal divisions. The outer layer of the o.i. and funicle may already start accumulating tannic substances before fertilisation has taken place (*fig. 15*). The curvation of the ovule exceeds 90°, but the ovule does not become campylotropous (*fig. 5 b*). The micropyle is ultimately formed by the i.i. The endostome is suborbicular. Starch is present in all parts of the ovule, but it is mainly concentrated in the apical parts of the i.i., o.i. and nucellus.

Development of seed and testa

During the post-fertilisation stage of development a marked growth in thickness of the integuments takes place, so that the i.i. and o.i. ultimately become 5–7 and 6–10 layers thick, respectively (*figs. 4 g, 16* and *17*). The nucellus is gradually resorbed, the part at the chalazal side remaining intact longest. The i.i. is also resorbed and squeezed flat before seed maturity (*fig. 17*). The cells of the o.i. are also compressed, but their walls remain as a fibrose layer. Cell
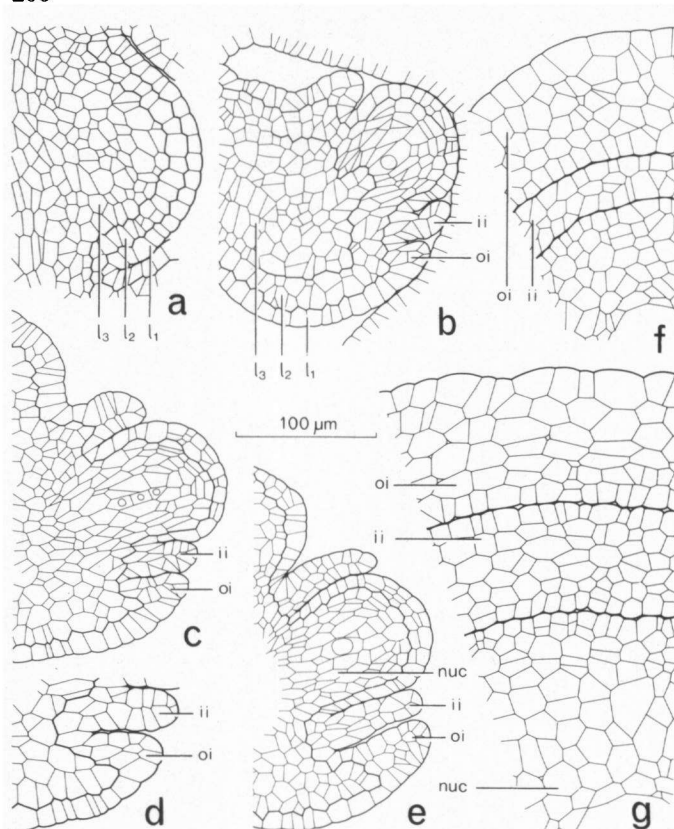
Fig. 4. *Skimmia japonica*. a, b, c, e: l. sns. of developing ovules; d: detail of l.s. of ovule corresponding with stage c (higher magnif.); f: detail of tr. s. of nearly full-grown ovule; g. do. of early post-fertilisation stage.

wall thickening does not take place. The outer dermal layer with its tannin cells remains intact longest and is recognisable from the concavely impressed cells in the mature testa (*figs. 18* and *21*). Owing to these developments the mature testa of *Skimmia* is of a relatively simple construction. From the periphery downwards the following layers are discernible (see *fig. 18*): the concave remains of the dermal cells of the o.i. (also *fig. 21*), the fibrous layer of compressed o.i. cells, the relatively thick nucellar cuticle, a thin layer consisting of the remains of nucellus and endosperm. The chalaza is somewhat broader than in the other two species; the funicle has a central amphicribral vascular bundle. The mature seed is light brown in colour, about $4 \times 4 \times 6$ mm (*fig. 19* and *20*); the seeds are often mutually flattened on their flanks. Tannic products located around the vascular tissue of the chalaza stain the seed coat a dark brown.
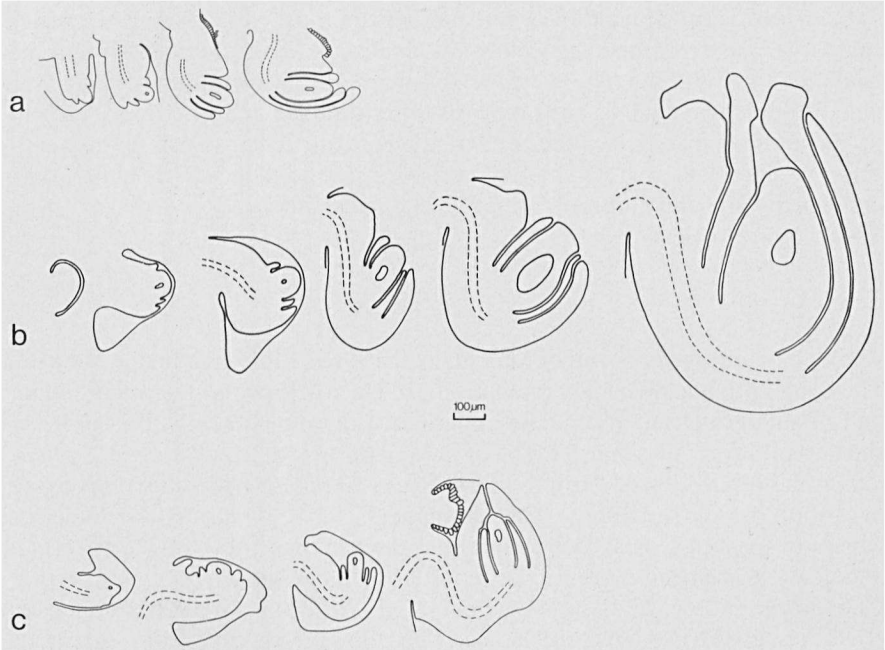
Fig. 5. Anatropism of the ovules of A: *Ruta graveolens*, B: *Skimmia japonica*, C: *Zanthoxylum simulans*, successive stages. Explanation in text.
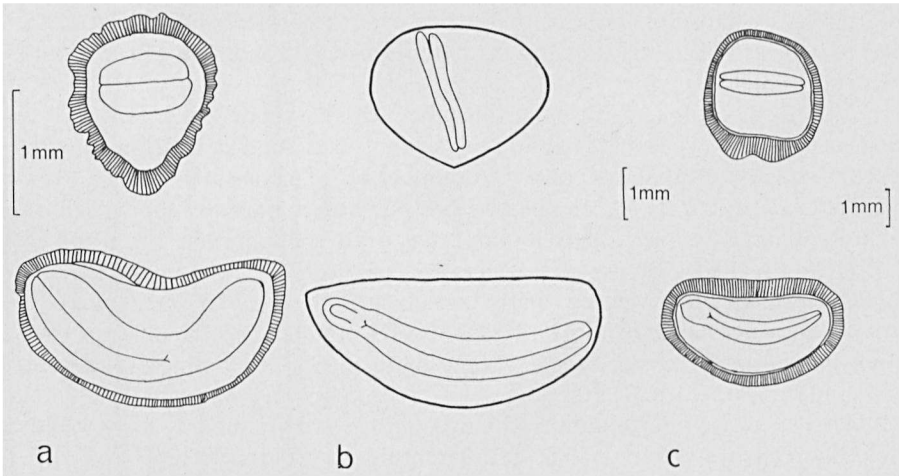
Fig. 6. L. (bottom row) and tr. sns. (top row) of rutaceous seeds. A: *Ruta graveolens*, B: *Skimmia japonica*, C: *Zanthoxylum simulans*.

Each seed is surrounded by a thin and bony, almost white stony layer which can rather easily be detached from the fruit wall. This stony layer may be mistaken for the testa. The seed coat proper is a thin membranous layer situated under the stony layer. The cotyledons and the endosperm are rich in lipids but poor in starch. The nucellar cuticle in particular is rich in fatty substances. The testa is not lignified and does not contain starch either. The endosperm and the embryo contain pectic substances.

4. DISCUSSION

The ovular ontogeny is rather similar in the three rutaceous taxa examined. The ovules are bitegmic and crassinucellate. The ovule primordia are trizonate and of subdermal initiation. The initiation of the integuments takes place in the dermal layer. The i.i. is always of dermal derivation. In *Ruta* and *Zanthoxylum* the o.i. is also of dermal origin, but in *Skimmia* also subdermal tissue contribute to its formation. The integuments in *Skimmia* (Toddalioideae), generally speaking, seem to be somewhat thicker than those of *Zanthoxylum* and *Ruta* (Rutoideae). An arillus, reported to be present in *Zanthoxylum* by CORNER (1976: pg. 232), was not observed. According to SCHLOTTERBECK (1896; see also NETOLITZKY 1926: pg. 178), the i.i. no longer forms a part of the mature testa. Also DESAI (1962) believed that in *Zanthoxylum* the i.i. is completely resorbed before seed maturity, but these ideas are erroneous. In contrast to the early development stages, the development and structure of the mature testa varies appreciably from species to species. The seed coat of *Zanthoxylum* is very hard owing to the presence of strongly sclerotised elements. In *Ruta* only the outermost cells of the testa have important cell wall thickenings, and in *Skimmia* the tegumentary derivatives degenerate. In the latter genus the solid endocarp has taken over the protective function of the testa (when the fruit is eaten and the pyrene passes through the digestive tract in endozoochory).

Among the Angiosperms, the bitegmic, crassinucellate ovule is primitive (BOUMAN 1974). The i.i. is almost invariably of dermal derivation, but the o.i. originally of subdermal origin. A number of rutaceous taxa such as *Skimmia*, *Poncirus*, *Citrus*, *Glycosmis* and *Choisya* have a partially subdermal o.i. (BOESEWINKEL, unpublished). If one takes into account that a number of reputedly related groups such as Geraniaceae, Oxalidaceae, Linaceae, Zygophyllaceae, Erythroxylaceae, and Malpighiaceae have outer integuments of exclusively dermal derivation, there is a solid argument in favour of a basic position of the Rutaceae in respect of the above-mentioned families (if the latter are indeed affined).

An alternative interpretation is that Rutaceae and Geraniales are not at all closely related and may belong to different major groups of dicots.

LITERATURE

BIERMANN, M. (1896): *Beiträge zur Kenntnis der Entwicklungsgeschichte der Früchte von Citrus vulgaris und anderer Citrus Arten.* Thesis. Zürich.

BOUMAN, F. (1974): *Developmental studies of the ovule, integuments and seed in some Angiosperms.* Thesis. Amsterdam.

CORNER, E. J. H. (1976): *The seeds of Dicotyledons.* Vol. 1 and 2. Cambridge.

DESAI, S. (1962): Polyembryony in Xanthoxylum Mill. *Phytomorphology* 12: 184–189.

ENGLER, A. (1964): *Syllabus der Pflanzenfamilien* II. Berlin.

—, & K. PRANTL (1931): *Die Natürlichen Pflanzenfamilien.* 2. Aufl. Bd. 19a. Leipzig.

FISH, F. & P. G. WATERMAN (1973): Chemosystematics of the Zanthoxylum/Fagara complex. *Taxon* 22(2.3): 177–203.

GALLET, F. (1913): *Développement et structure anatomique du tégument séminal des Rutacées.* Thesis. Paris.

HARTL, D. (1958): Struktur und Herkunft des Endocarpes der Rutaceen. *Beitr. Biol. Pflanzen* 34: 35–49.

HEGNAUER, R. (1971): Chemical patterns and relationships of Umbelliferae. In Heywood, V.H. (ed.): *The biology and chemistry of the Umbelliferae. Suppl. y. Linn. Soc. Bot.* 64: 267–277.

MEEUSE, A. D. J. (1970): The descent of the flowering plants in the light of new evidence from phytochemistry and from other sources. I and II. *Acta Bot. Neerl.* 19: 61–72, 133–140.

NETOLITZKY, F. (1926): *Anatomie der Angiospermen-Samen.* In: K. Linsbauer (ed.) Handbuch der Pflanzenanatomie. Band X. Berlin.

SASS, J. E. (1958): *Botanical microtechnique.* Third. ed. Ames, Iowa.

SCHLOTTERBECK, J. O. (1896): *Beiträge zur Entwicklungsgeschichte pharmakognostisch wichtiger Samen.* Thesis. Bern.

TAKHTAJAN, A. (1973): *Evolution und Ausbreitung der Blütenpflanzen.* Stuttgart.

WATERMAN, P. G. (1975): Alkaloids of the Rutaceae: their distribution and systematic significance. *Biochem. Syst. Ec.* 3: 149–180.

Fig. 7–10. *Ruta graveolens.*
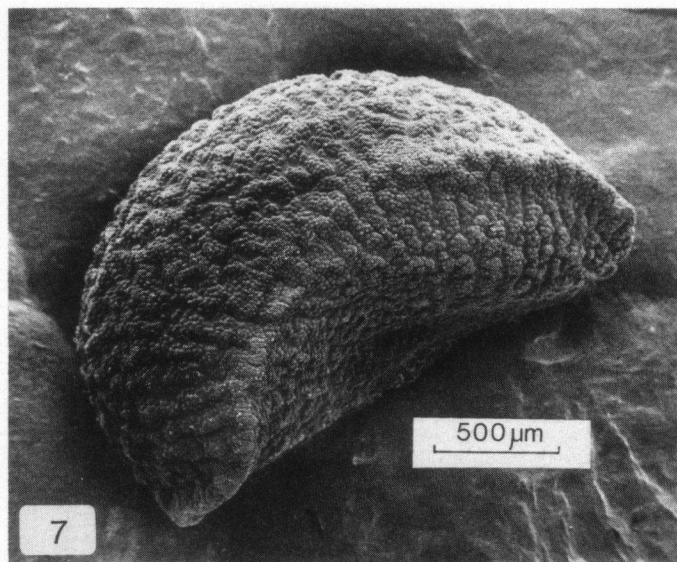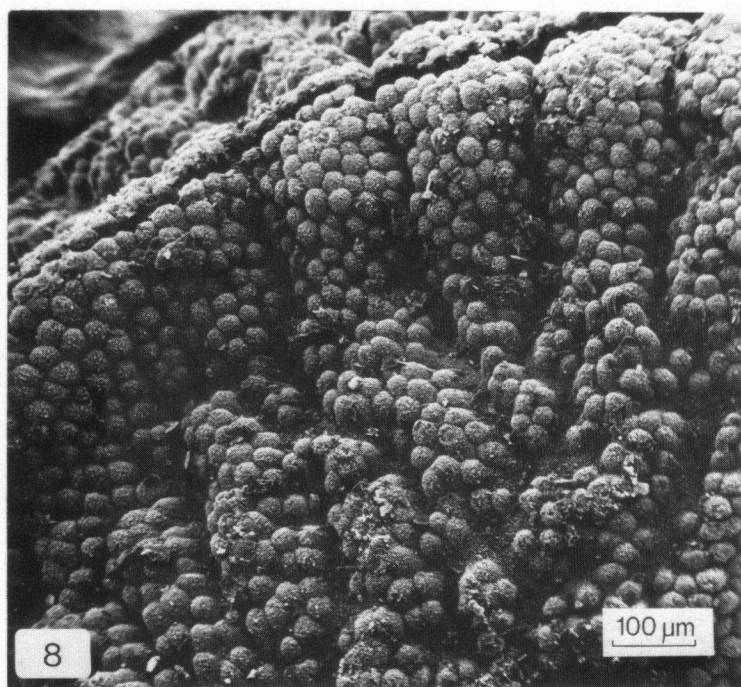

Fig. 7. 7: whole seed. SEM photograph.

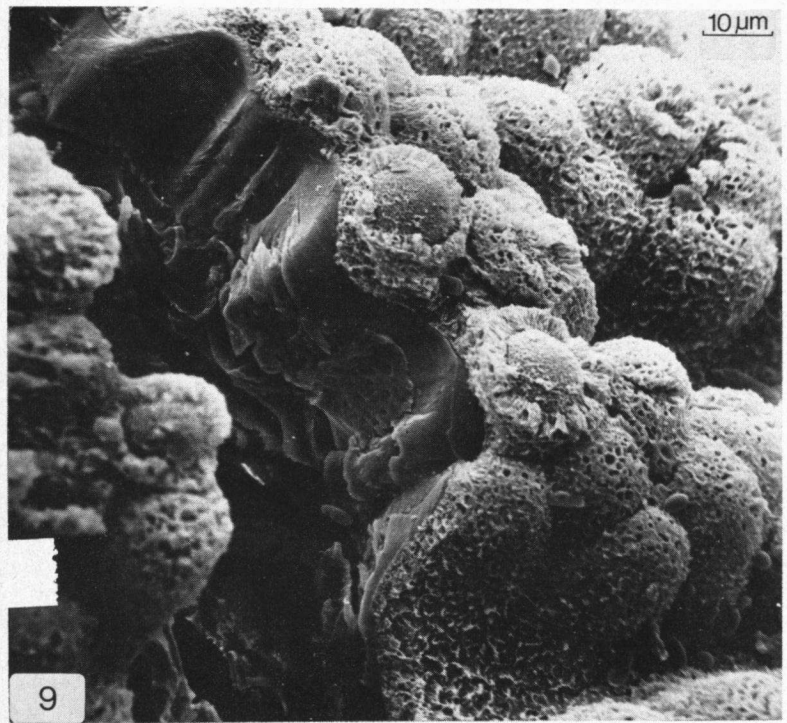Fig. 8: detail of testa, surface view. SEM.
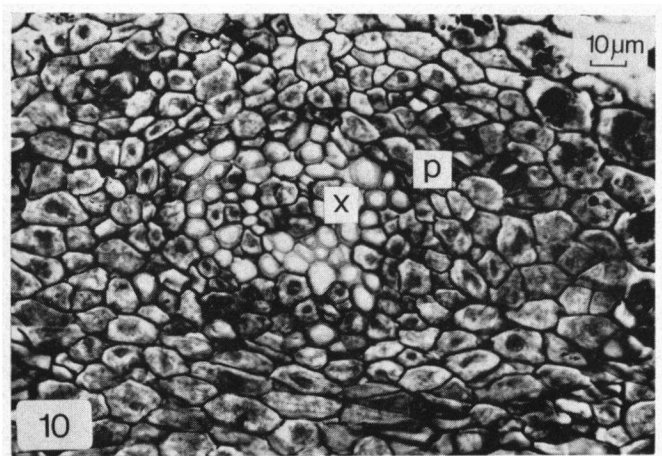
Fig. 9: fissure in testa. SEM.

Fig. 10: transmitted light photomicrograph of tr. s. of funicle showing amphicribal vascular bundle, X = xylem, P = outer phloem.
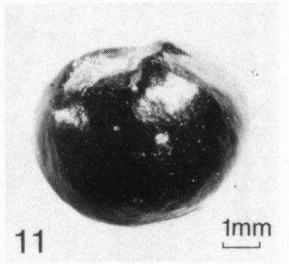
Fig. 11–13. *Zanthoxylum simulans.*

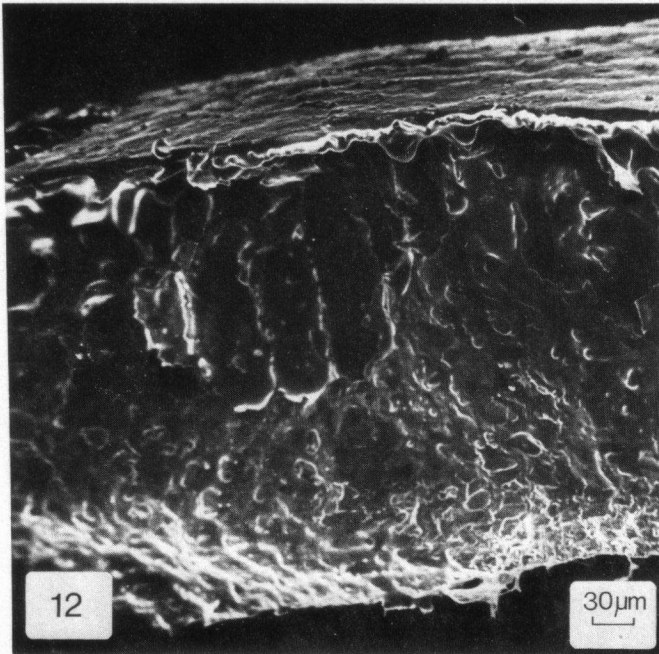Fig. 11: ripe seed. Photomacrograph.
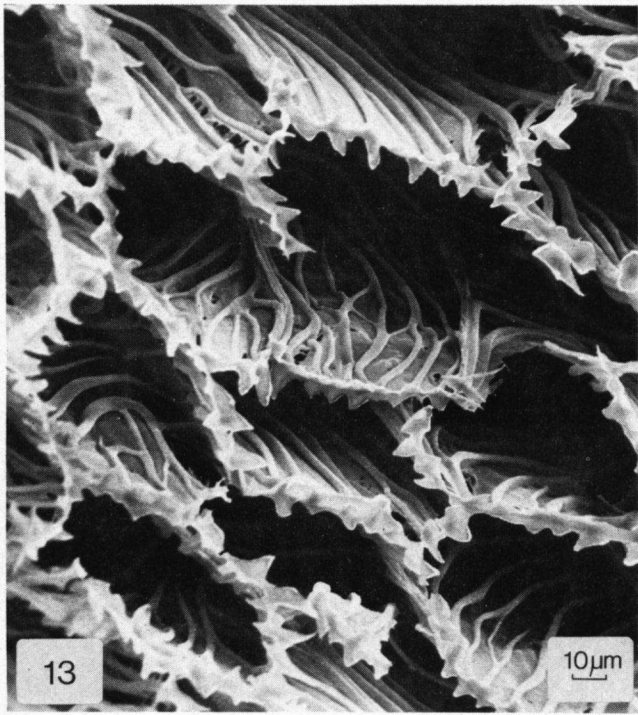

Fig. 12: tr. s. of testa. SEM.

Fig. 13: thickened cell wall ridges in cells of i.i. SEM.

Fig. 14–21. *Skimmia japonica*, photomicrographs, and SEM photographs.
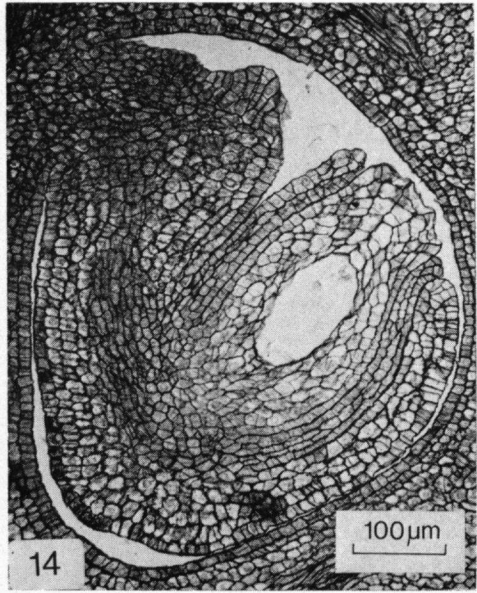
Fig. 14: l. s. of almost mature ovule.
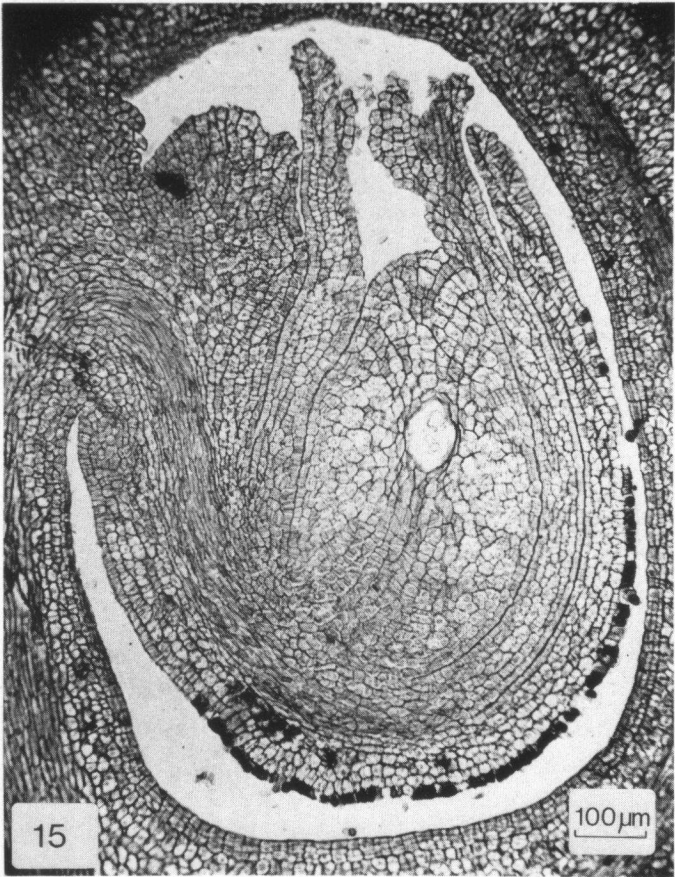
Fig. 15: l. s. of mature ovule.
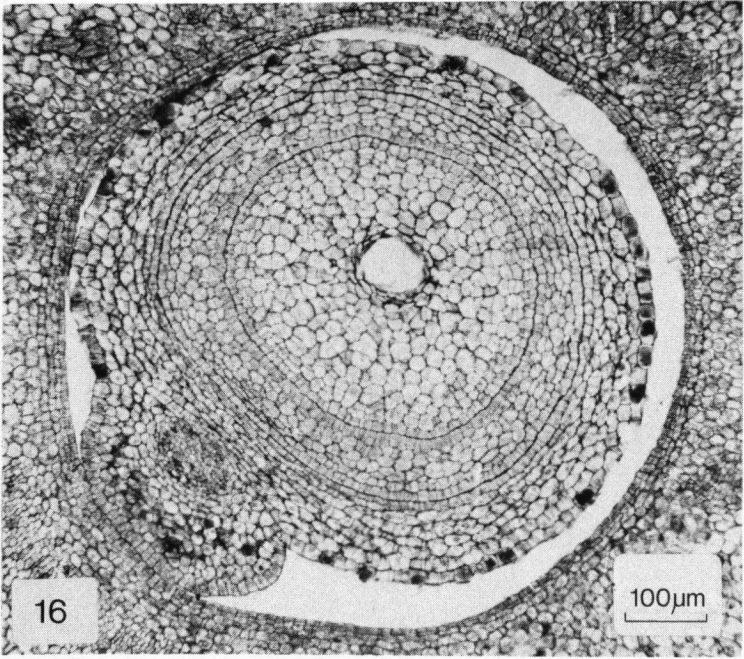
Fig. 16: tr. s. of same.
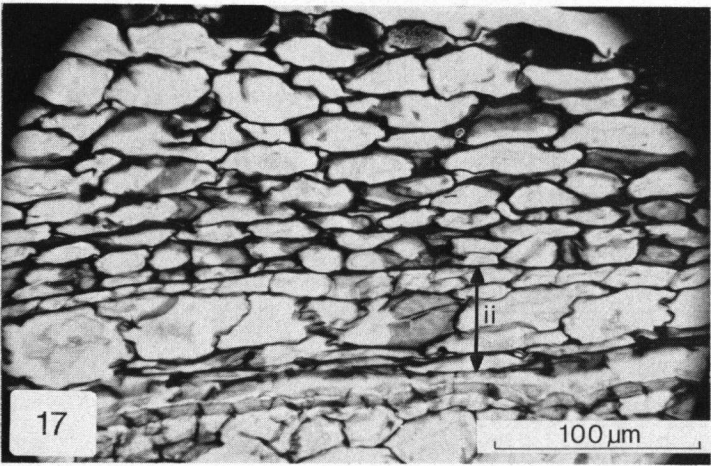
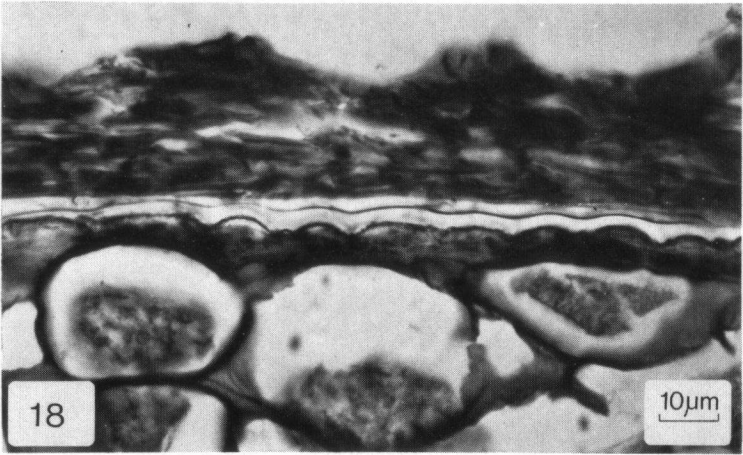
Fig. 17: tr. s. of testa during the resorption of the i.i.
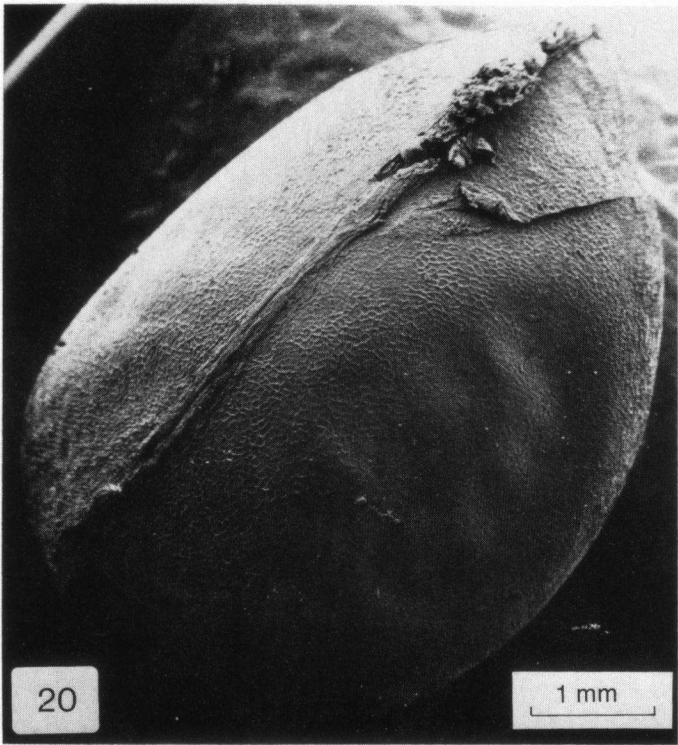
Fig. 18: tr. s. mature seed coat.
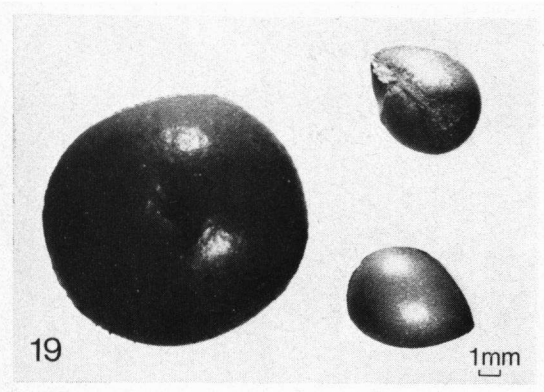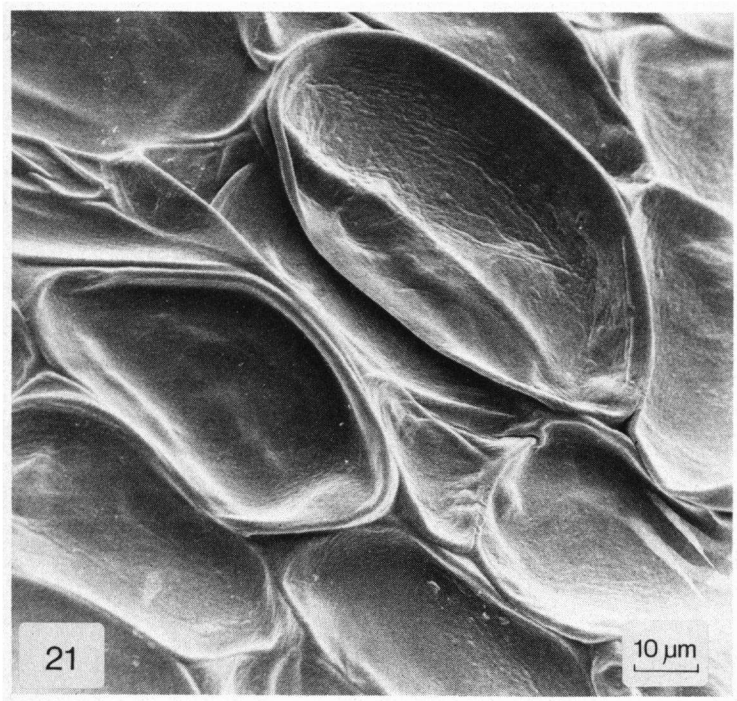

Fig. 20: whole seed. SEM.

Fig. 19: photomacrograph of two seeds and a fruit.

Fig. 21: detail of testa surface. SEM.