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A DEVELOPMENTAL STUDY OF THE INTEGUMENT IN GYMNOSPERMS 3. EPHEDRA DISTACHYA L. AND E. EQUISETINA BGE.

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SUMMARY

The early ontogeny of the two ovular envelopes of *Ephedra distachya* and *E. equisetina* was thoroughly studied as a part of the comprehensive investigations on the integument and associated structures in Gymnosperms. The outer envelope is initiated as a horseshoe-shaped primordium at the ventral (or axial) and lateral sides to become continuous later on; this envelope grows out more rapidly at the lateral sides, so that the young envelope appears as two opposite projections; the mature envelope is a stout structure vascularized at the lateral sides. The inner envelope, which is initiated a little later than the outer one, arises as a ring-shaped primordium and elongates, more conspicuously so at the ventral side, and finally forms a long micropylar tube. Although the greater part of the inner envelope is of a uniform thinness, the basal part swells up appreciably. Histologically the outer envelope is formed by derivatives of both the dermal and the subdermal cells of the ovule primordium (i.e., it is of dual origin), but the inner envelope exclusively by derivatives of the dermal cells except in its basal part (i.e., it is of dermal origin). Evidence from the morphology and anatomy does not support the homology between the two envelopes and suggests that the outer envelope resembles vegetative leaves more than the inner one. The possible numbers and arrangements of the constituting elements of the envelopes are also discussed.

1. INTRODUCTION

The female reproductive structure (ovule) of *Ephedra* characteristically bears two envelopes (an outer and an inner one) which enclose a nucellus at the time of pollination. A considerable number of authors have studied the inner (ie) and outer (oe) envelopes macromorphologically (including the ontogenetic stages: HAGERUP 1934; LEHMANN-BAERTS 1967a), anatomically (including the histogenesis and vascular anatomy: VAN TIEGHEM 1869; STRASBURGER 1872, 1879; JACCARD 1894; THODAY & BERRIDGE 1912; HERZFELD 1922; PEARSON 1929; MAHESHWARI 1935; EAMES 1952; SEELIGER 1954; PANKOW 1962; SINGH & MAHESHWARI 1962; LEHMANN-BAERTS 1967b; FAGERLIND 1971) and teratologically (MEHRA 1950), and, furthermore, in relation to the embryology (LAND 1904, 1907; BERRIDGE & SANDAY 1907; LEHMANN-BAERTS 1967c). The organographic nature and homology of both the envelopes thus have been discussed in various ways. Their interpretation, although not always clearly expressed, varies: (1) the oe has been interpreted as a carpellary organ and the ie as an integument

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(VAN TIEGHEM 1869, 1891; STRASBURGER 1872), (2) both envelopes are integuments (STRASBURGER 1879; LAND 1904, 1907; THODAY & BERRIDGE 1912; MEEUSE 1978); LAND (1904) considered them to be foliar in nature, (3) the oe is a leafy organ similar to bracts, bracteoles or a perianth member, and the ie is an integument (JACCARD 1894; ARBER & PARKIN 1908; PEARSON 1929; MEHRA 1950; EAMES 1952; LEHMANN-BAERTS 1967a; FAGERLIND 1971; FOSTER & GIFFORD 1974), (4) the oe and ie are both leafy organs, and the ie is a carpellate structure (LIGNIER & TISON 1911), (5) the oe is an organ comparable to the "Fruchtschuppe" of Taxaceae, and the ie is an integument (HERZFELD 1922) or (6) the oe consists of prophylls, and the ie is a sporophyll (HAGERUP 1934). The envelopes have been also controversial as regards the number and arrangement of their constituents. For instance, the oe has been interpreted as consisting of (1) two elements (viz., a dorsal, or bracteal, and a ventral, or axial, one) in the median plane (VAN TIEGHEM 1869; EAMES 1952), (2) two such elements of the lateral plane (STRASBURGER 1872; VAN TIEGHEM 1891; JACCARD 1894; PEARSON 1929; HAGERUP 1934; MEHRA 1950), (3) four elements inserted at the same level (LAND 1904, 1907) or (4) one dorsal and two laterals (LIGNIER & TISON 1911). In a similar way the ie has been considered to comprise (1) two of an uncertain (LAND 1904, 1907) or of a probable lateral orientation (EAMES 1952), (2) one ventral and two dorsal elements (LIGNIER & TISON 1911) or (3) one ventral element (HAGERUP 1934). These various opinions have been reviewed by STRASBURGER (1872), THODAY & BERRIDGE (1912), PEARSON (1929), LEHMANN-BAERTS (1967a) and MARTENS (1971).

None of the previous authors have thoroughly documented the ontogeny of the envelopes under discussion, and possibly the above-mentioned conflicting opinions are attributable to inadequate information. In this paper I present a complete record of the morphological and anatomical features of the envelopes during their ontogeny. This study is intended to solve the controversy regarding the organographic nature of the envelopes.

2. MATERIALS AND METHODS

Ephedra has one to three female reproductive organs (ovules) per strobilus depending on the species. This paper treats two species bearing two of them: *Ephedra distachya* L. and *E. equisetina* Bge. The species are being cultivated at Tsukuba Medical Plant Research Station, National Institute of Hygienic Sciences in Japan; the voucher specimens are preserved at the Makino Herbarium (MAK 189060, 189061). Female strobili in various stages of development were collected from early to late May and were fixed with formalin-acetic acid-alcohol (FAA). Observations were made by light microscopy and scanning electron microscopy (SEM). Some of the fixed materials were dehydrated through a tertiary-butyl alcohol series, embedded in paraplast and sectioned 10 μm in thickness using a rotary microtome. The sections were stained with Heidenhain's haematoxylin, safranin and fast green. For SEM observations, some other materials were de-

hydrated in an ethyl alcohol series, dried in a critical point dryer with carbon dioxide, and coated with gold.

Descriptive notes: *Ephedra distachya* and *E. equisetina* are small dioecious shrubs. A female plant of *E. distachya* usually produces three strobili from each axil of decussately arranged foliage leaves, while that of *E. equisetina* forms only one such reproductive shoot. The apical part of the strobili produces as a rule four pairs of bracts arranged decussately, and the upper pair (marked *br.* 4 and *br.* 4′ in the figures) subtend the reproductive organs consisting of a nucellus and its two envelopes (*figs. 1* and *2A, B*). For the sake of convenience, a female reproductive organ is referred to as an "ovule" in this paper.

LEHMANN-BAERTS (1967a) gave descriptions of the ovule by observations from four sides: ventral side, both lateral sides and dorsal side (*fig. 1A*; cf. FAGERLIND 1971; SINGH 1978). The terms are also used here for the descriptions of parts of the envelopes, for example: the ventral side of the ie. The ventral side faces the axis of the strobilus, and is the portion farthest away from the midrib of the uppermost bract subtending the ovule; the dorsal side is at the bracteal side, thus the portion nearest to the midrib of the bract; the lateral sides refer to the opposite portions between the ventral and dorsal sides (*fig. 1*).

Descriptions of histological features have mainly been based on median and transmedian longisections of the ovule. The median longisection runs through the ventral and dorsal sides as well as the apex of the ovule (*fig. 1B, D*); and the transmedian longisection through the lateral sides and also through the apex of the ovule (*fig. 1C, E*).

3. OBSERVATIONS

Barring a few minor points, the ovules of *Ephedra distachya* and of *E. equisetina* show basically the same developmental features. The following descriptions are common to both species unless otherwise mentioned.

Reproductive axis and ovule primordium: The characteristics of the reproductive axis and ovule primordium in both species (see TAKASO, in press) agree with previously published descriptions of the ovule of *E. distachya* (THODAY & BERRIDGE 1912; HAGERUP 1934) and of other species (STRASBURGER 1872, 1879; HERZFELD 1922; MAHESHWARI 1935; EAMES 1952; SEELIGER 1954; PANKOW 1962; FAGERLIND 1971).

At each axil of the uppermost bracts of a reproductive axis, an ovule primordium arises as a relatively large, semiglobose outline (*figs. 2A* and *3A*). The ovule primordium is oriented in the oblique direction departing from the reproductive axis by nearly 45 degrees (but ultimately the mature ovules come to stand upright and parallel with the reproductive axis, see *fig. 2H*). At the apical part of the reproductive axis a furrow is formed between the twin ovule primordia, and at the summit of this furrow the residual shoot apex is discernible as a small protuberance. Despite its residual nature, this small outgrowth may develop into a somewhat prominent structure.

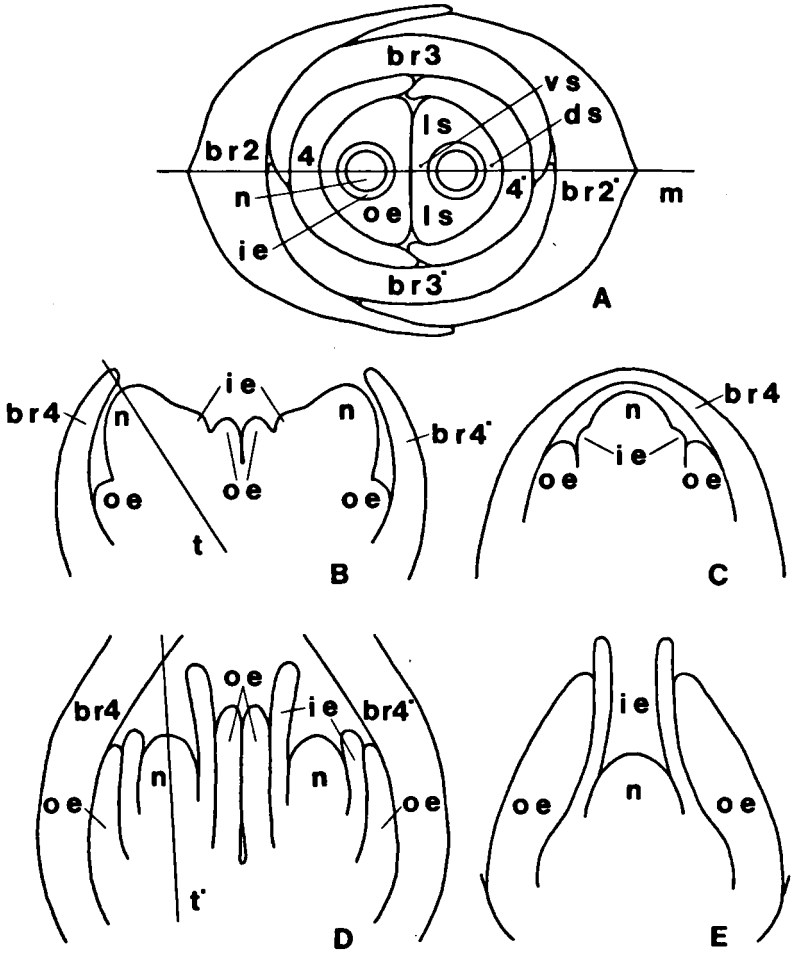

Fig. 1. Diagrammatic illustrations of the female strobilus showing the relation of ovules and associated bracts. A: Cross section of ovules at the middle stage. An ovule is divided into dorsal, ventral and lateral sides (ds, vs and ls). B, D: Median longisections (marked m in A) of ovules at the early and middle stages. C, E: Transmedian longisections of ovules at the lines t, t' in B and D. Br 2, 2', 3, 3', 4, 4' are decussately arranged pairs of bracts, and br 4, 4' indicate the uppermost pair. ie: inner envelope; n: nucellus; oe: outer envelope.

Histologically the ovule primordium shows a remarkable two cell-layered superficial structure except at the dorsal side, a condition which has clearly resulted from regular periclinal cell divisions in the original dermal layer (*fig. 3A*). Below these two cell layers both periclinal and anticlinal cell divisions repeatedly occur and cause a rapid growth of the ovule primordium.

Initiation of the oe: According to STRASBURGER (1872, 1879) and HAGERUP (1934) the oe is initiated at two places in the lateral plane of symmetry at sites a little

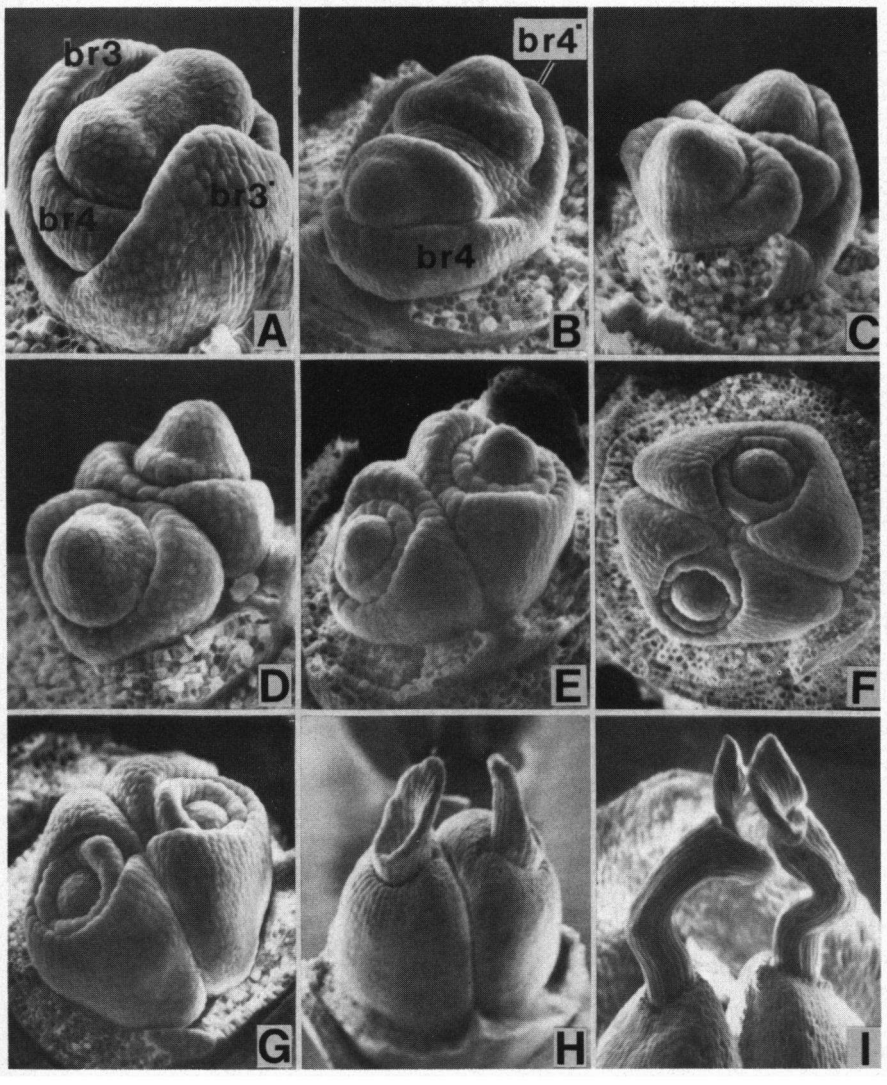

Fig. 2. Scanning electron micrographs of ovules at various stages of development (A–D, F and G: *E. distachya*; E, H and I: *E. equisetina*). A: The stage when ovule primordia are swelling, × 115. B: The stage when the oe is being initiated, × 100. C, D: The stage when the ie is being initiated, × 100, 85. E, F and G: The middle developmental stages of ovules, × 75, 70, 70. H, I: Almost mature stages, × 50, 40. br 3, 3', 4, 4': pairs of bracts.

shifted to the ventral side, in *E. campylopoda* and *E. distachya,* respectively. According to my findings, however, the oe arises from all over the base of the ovule primordium except at the dorsal side (*figs. 2B, 3B* and *4A*). Therefore,

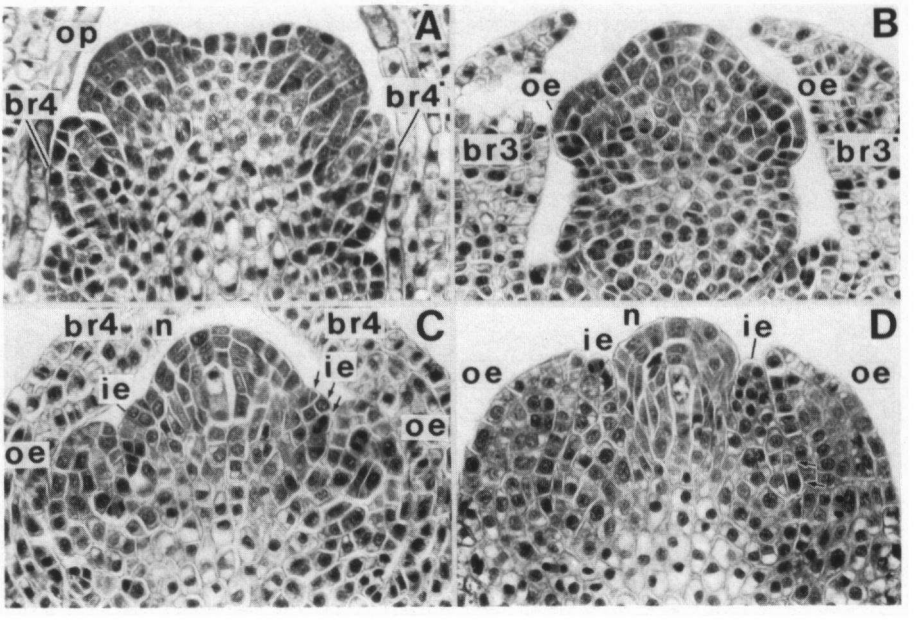

Fig. 3. Successive developmental stages of young ovules (A–C: *E. distachya*; D: *E. equisetina;* × 160). A: Median longisection of ovule primordia (op). B, C: Transmedian longisections at the stages of the initiation of the oe and ie. D: Transmedian longisection showing the basal swelling of the ie. Arrows indicate periclinal cell divisions in the dermal layer. br 3, 3', 4, 4': pairs of bracts; ie: inner envelope; n: nucellus; oe: outer envelope.

the early swelling of the oe is horseshoe-shaped, as observed in *E. americana* by PANKOW (1962) and in several species by FAGERLIND (1971).

In both transmedian and median longisections, the bulging oe has a two-layered structure, a feature also characteristic of the ovule primordium (*figs. 3B* and *4A*). Cells of the outermost layer sometimes divide anticlinally forming the secondary dermal layer and covering the second layer, i.e., the secondary subdermal layer. Also cells just below the two-layered structure divide mainly in the periclinal plane contributing slightly to the swelling, especially at the lateral sides (*figs. 3B* and *4A*; compare PANKOW 1962).

Early development of the oe: As the ovule develops, the oe becomes discernible at the dorsal side as well, so that the oe becomes a continuous structure (compare STRASBURGER 1872, 1879; HERZFELD 1922; HAGERUP 1934; FAGERLIND 1971). In the dorsal swelling of the oe, the two-layered structure is not so regular as it is at the other sides (*fig. 4B*; PANKOW 1962). By this stage, the oe forms two remarkable projections on its rim, which appear to be positioned at the "true" lateral sides as described in *E. helvetica* by JACCARD (1894), and not at the lateral sides a little to the ventral side as STRASBURGER (1872, 1879) and PEARSON (1929) reported (see *fig. 2C, D*).

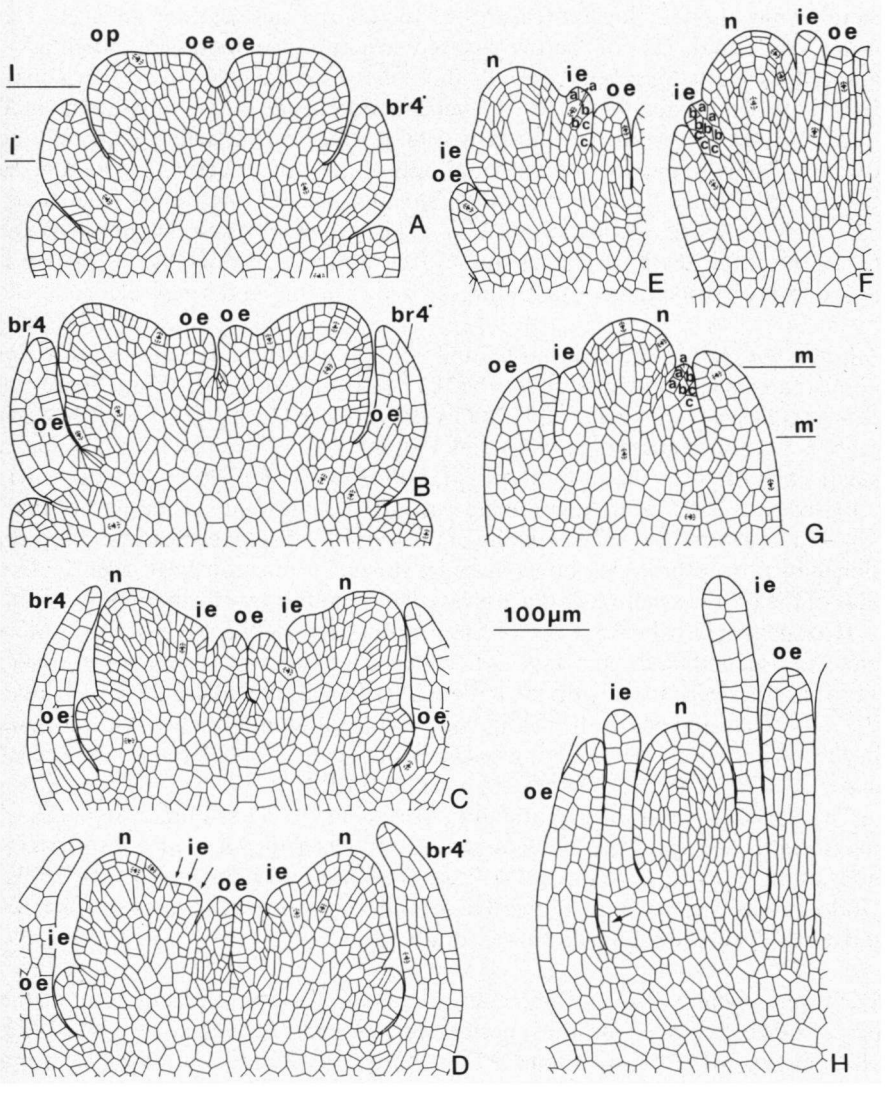

Fig. 4. Ovules at the early and middle stages (A–D: *E. distachya*; E–H: *E. equisetina*). A–D: Median longisections of ovules at the stages of the initiation of the oe and ie. E–G: Median and transmedian longisections showing the mother cells of the ie. Derivatives from the same mother cells are marked with the same letters (a, b, c). H: Median longisection of an ovule at the middle stage. Lines marked 1, 1', m, m' indicate the junction of two ovule primordia, the ovule primordium-bract junction, nucellus-ie junction and ie-oe junction, respectively. Arrows point out periclinal or oblique cell divisions in the dermal layer. br 4, 4': uppermost bracts; ie: inner envelope; n: nucellus; oe: outer envelope; op: ovule primordium.

Although the growth rate of the oe varies at its different sides even in the

same ovule, the histological features of the oe are quite similar all over. The outer, or dermal, cells of the two-layered structure usually divide anticlinally, and cells beneath this layer, especially those in the second layer, divide periclinally or somewhat irregularly (see the ventral side in *fig. 4B, C*; the dorsal side in *fig. 4C, D*; and compare PANKOW 1962). Later on, periclinal and oblique divisions come to prevail in the dermal cells of the apical region all around (see the ventral side in *fig. 4D*; the lateral sides in *fig. 3C*; the dorsal side in *fig. 4E*; and compare FAGERLIND 1971). The growing oe is slightly thicker (5–7-layered) at the lateral sides than at the ventral and dorsal sides (where it is 4- or 5-layered) and has longitudinal cell-rows in the subdermal region.

Initiation of the ie: The external features of the ie at the stage of initiation are almost the same as previously described by STRASBURGER (1872, 1879), BERRIDGE & SANDAY (1907), HERZFELD (1922), PEARSON (1929), HAGERUP (1934), PANKOW (1962), LEHMANN-BAERTS (1967a) and FAGERLIND (1971). The ie appears as a small swelling after the initiation of the oe (*fig. 2C, D*). More precisely, it is initiated at a little later stage or almost at the same time as the oe becomes visible at the dorsal side. The bulging of the ie usually begins earlier at the ventral side than at the other sides, but sometimes starts simultaneously all over. Unlike that of the oe, the swelling of the ie is annular from the very beginning.

Histogenetically there are two or three mother cells of the ie lying in the outermost, i.e., secondary dermal, layer when seen in longisections of the young ovule. These mother cells usually divide periclinally. In the case where there are three, the cells above and below the median one divide rather obliquely (see the arrows in *figs. 3C* and *4D*). The bulging results from such divisions (compare STRASBURGER 1872, 1879; PANKOW 1962; FAGERLIND 1971).

The nucellus is outlined by the initiation of the ie. The constituting cells of the nucellus are upon the whole arranged fanwise (*fig. 4E, F*; see also STRASBURGER 1872, 1879; JACCARD 1894; LEHMANN-BAERTS 1967c; FAGERLIND 1971). Undoubtedly these cells are derivatives of cells of the outer two layers so characteristic of the ovule primordium.

Early development of the ie: The ie grows faster at the ventral side than at the dorsal side as reported in other species (*figs. 2E* and *4E, F*; STRASBURGER 1872, 1879; PEARSON 1929; FAGERLIND 1971). All the constituting cells of the growing ie are derivatives of the cells of the initial swelling. As a rule cell divisions are oriented in the same direction as in the mother cells, so that two (as STRASBURGER 1872, observed in *E. campylopoda*) or three longitudinal cell-rows are formed (see the cells marked *a, b* and *c* in *fig. 4F, G*). In the case of three cell-rows, the cells of the outermost one (see the cells marked *c* in *fig. 4E–G*) divide less frequently than those of the other rows. Thus the greater part of the ie, except its lower portion, becomes two cell-layered (*figs. 3D* and *4H*).

Active divisions occur in the dermal and subdermal cells at the junction of the young oe and the bulge of the ie as described in *E. distachya* and other species by STRASBURGER (1872, 1879), THODAY & BERRIDGE (1912) and FAGERLIND

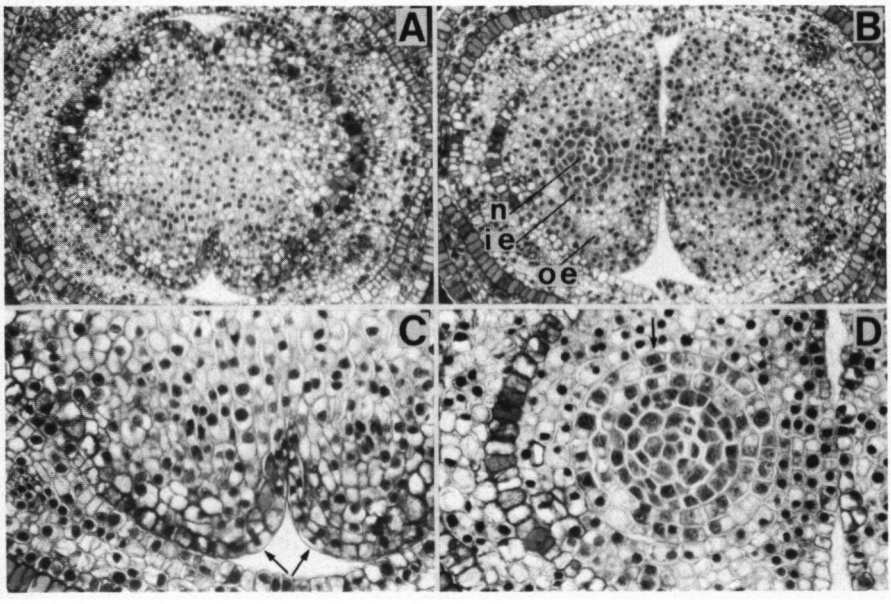

Fig. 5. Cross sections of a relatively developed ovule (*E. equisetina*). A, B: At the levels of the lower and middle parts of the ovule, × 75. C, D: Details of A and B, × 155. Arrows indicate periclinal cell divisions. ie: inner envelope; n: nucellus; oe: outer envelope.

(1971). This growth also takes place in the outer cell-row (marked *c* in *fig. 4E–G*) of the ie. As a result, the basal part of the ie considerably extends in the radial direction, especially at the lateral sides (*figs. 3D* and *4G*). Furthermore, the distance between the nucellus-ie junction (marked *m* in *fig. 4G*) and the ie-oe junction (marked *m'* in *fig. 4G*) becomes fairly great.

Growth of the oe: During the later stages of development the apex of the ovule turns in a more distal direction and eventually comes to lie parallel to the elongated ovule-bearing axis. In cross sections the ovule is plan convex (*fig. 5A, B*), and twin ovules come into contact with one another along their ventral sides. At this developmental stage the apices of the two projections of the oe have shifted from the true lateral sides towards the ventral side (*fig. 2E, F*; compare STRASBURGER 1872; PEARSON 1929; LEHMANN-BAERTS 1967a; FAGERLIND 1971). The growth of the ventral side of the oe is suppressed, and a cleft is formed here (*fig. 2F, G*). STRASBURGER (1872, 1879) described two clefts at the dorsal and the ventral sides in *E. campylopoda* (cf. MEHRA 1950), but such is not the case in the two species investigated.

The oe grows by elongation and division of every constituent cell. Most of the dermal cells of the growing oe divide anticlinally, i.e., in the same direction as the growth of the oe, but the apically situated cells tend to divide irregularly (*figs. 4H* and *6A*). Moreover, cells at the lower part sometimes divide periclinally (see the arrows in *fig. 5C*). The subdermal cells as well as the inner cells of the

oe divide anticlinally and periclinally (*fig. 6A*). A provascular strand differentiates within both lateral sides.

Growth of the ie: The ie grows beyond the nucellar apex. In contrast to the oe, the development of the ie is more pronounced at the ventral side (*fig. 2F, G*; STRASBURGER 1872, 1879; BERRIDGE & SANDAY 1907; PEARSON 1929; HAGERUP 1934; LEHMANN-BAERTS 1967a; FAGERLIND 1971).

The basal part of the ie is thick and massive all around but particularly at the lateral and dorsal sides. In this part periclinal cell divisions are common in both the dermal layer and the subdermal region, by which the basal swelling continues to develop (see the arrow in *fig. 4H*; see also *fig. 6A*), but these periclinal divisions occur relatively less frequently during the later stages of development. Probably the basal part of the ie is derived from cells constituting the two-layered structure in the ovule primordium.

The upper part of the ie is usually two cells thick (*figs. 4H* and *6A*), and this build-up is well retained up to the later stages of development. Apically situated cells may divide bifacially (*figs. 3D* and *4H*; FAGERLIND 1971), and the other cells divide anticlinally in respect of the surface of the ie. Periclinal divisions may also occur to form a three-layered portion at places (see the arrow in *fig. 5D*).

Mature ovule: The micropyle is formed by the ie alone (*fig. 2I*). The ie ultimately extends far beyond the oe to form a long micropylar tube with an elliptic orifice as reported by some previous authors (*fig. 2H, I*; STRASBURGER 1872, 1879; LAND 1904; HERZFELD 1922; PEARSON 1929; HAGERUP 1934; MAHESHWARI 1935; LEHMANN-BAERTS 1967a, b, c; FAGERLIND 1971; etc.). This opening is oriented towards the dorsal side, though the micropylar tube itself is irregularly twisted on the way. A tendency to coil is more conspicuous in *E. equisetina* (*fig. 2I*) than in *E. distachya*. The ie is thin, the lower part being only five cells thick at the most, and the upper part only two (STRASBURGER 1872; JACCARD 1894; LAND 1904; HERZFELD 1922; MAHESHWARI 1935; LEHMANN-BAERTS 1967a, b, c) or three cells thick (THODAY & BERRIDGE 1912; PEARSON 1929).

The oe encloses the ie, the micropylar tube excepted (*fig. 2H, I*). The cleft of the oe which was present at the ventral side in the previous stages gradually becomes obscure, because the mitotic activity and cell growth are comparatively slow at the apical part. The oe is a massive structure throughout its whole length (STRASBURGER 1872, 1879; JACCARD 1894; LAND 1904; PEARSON 1929; MAHESHWARI 1935; MEHRA 1950; LEHMANN-BAERTS 1967a, b, c; etc.). The upper and lower parts of the oe are five to nine cells thick at both the ventral and dorsal sides and about 20 or 30 cells thick at the lateral sides (*fig. 6C, E*).

In the two species studied the histological features of the mature ie and oe are essentially the same as those reported in *E. distachya* and in other species by STRASBURGER (1872, 1879), JACCARD (1894), THODAY & BERRIDGE (1912), HERZFELD (1922), PEARSON (1929), MAHESHWARI (1935), MEHRA (1950), SINGH & MAHESHWARI (1962), LEHMANN-BAERTS (1967a, b, c) and FAGERLIND (1971).

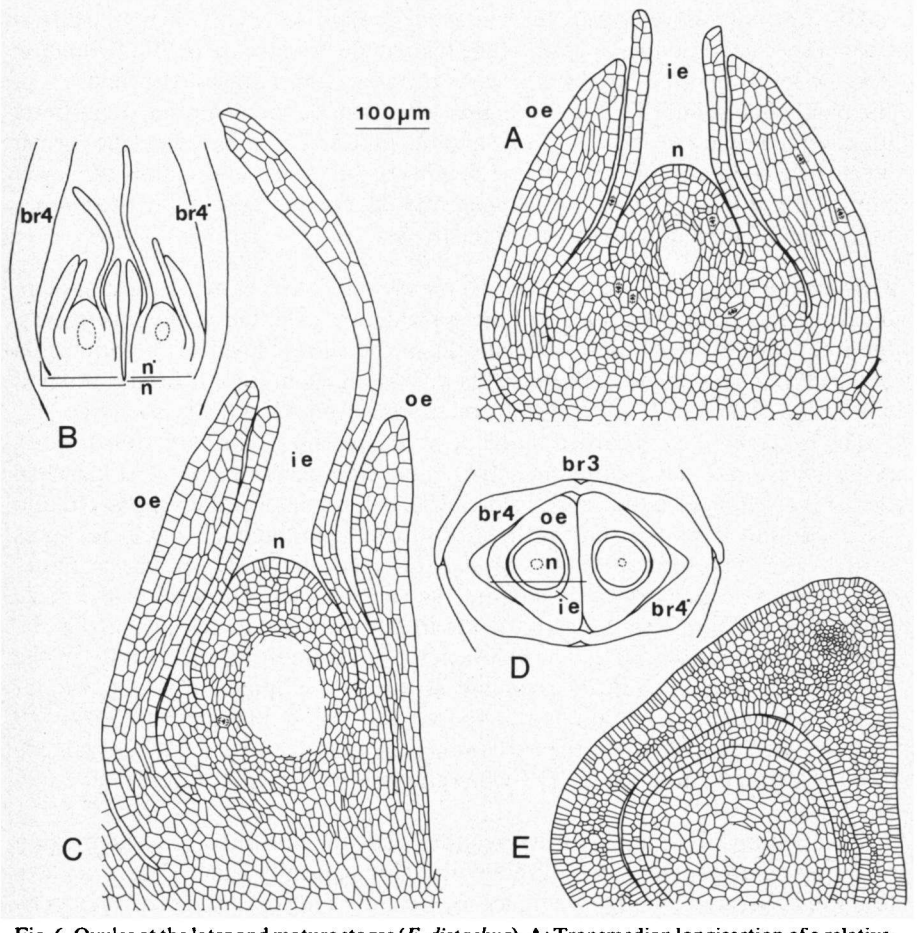

Fig. 6. Ovules at the later and mature stages (*E. distachya*). A: Transmedian longisection of a relatively developed ovule. B, D: Median longisection and cross section of mature ovules. C, E: Details of B and D. Lines marked n, n' indicate the junction of two ovules and the ovule-bract junction. br 3, 4, 4': bracts; ie: inner envelope; n: nucellus; oe: outer envelope.

They can be summarized as follows (see *fig. 6B–E*): As regards the oe, (1) most of the epidermal cells developed out of the dermal cells are large and longitudinally elongate; (2) the subdermal and inner cells of the lower part are also longitudinally stretched, whereas those of the upper part are enlarged and sometimes sclerotic; (3) the inner epidermal cells of the apical part assume the appearence of papillae; (4) most cells constituting the oe are tanniniferous; and (5) a vascular strand is present within each of the lateral sides. As regards the ie, on the other hand, (1) the cells of the upper part are relatively large, their size decreasing gradually towards the lower part; and (2) in the upper part the outer epidermal cells are longitudinally elongate, and the inner epidermal ones are enlarged and have a great affinity to stains.

During ovular development a remarkable change occurs involving the difference in level between the junction of the twin ovules and the ovule-bract junction (see the levels marked *l* and *l'* in *fig. 4A*). In the earlier stages, the junction of the twin ovule primordia lies at a higher level than the ovule primordium-bract junction (compare *l* with *l'* in *fig. 4A*); in the mature stage, however, the former level is situated at almost the same height as, or rather lower than the latter one (compare *n* and *n'* in *fig. 6B*). Apparently this change coincides with a change in the general direction of growth of the ovules.

Frequency of periclinally divided cells in the dermal layer: As an attempt to demonstrate histogenetic features of the reproductive structure in Gymnosperms, the frequency of periclinally divided cells in the dermal layer of the primordia of the two envelopes and of the nucellus was examined in *Ephedra* and compared with those in *Ginkgo* (TAKASO 1980) and in *Pinus* and *Abies* (TAKASO 1981).

The frequency was recorded in the oe at the ventral, lateral and dorsal sides, respectively, and in two different stages of development; viz., at the stage when the oe is swelling (see the ventral side in *fig. 4A*; the lateral sides in *fig. 3B* and the dorsal side in *fig. 4B*), and at the stage when the initial bulge of the oe begins to grow (see the ventral side in *fig. 4B*; the dorsal side in *fig. 4C, D*). The examination of the ie was likewise done from the same angles, but only in the stage when ie is swelling (see the ventral side in *fig. 4C*; the lateral sides in *fig. 3C* and dorsal side in *fig. 4D*). The frequency of periclinal mitotic activity in the nucellus was examined at its apex and in three developmental stages; viz., at Stage I, when the oe is initiated at the ventral and lateral sides (see *figs. 3B* and *4A*), at Stage II, when the oe commences to bulge also at the dorsal side (see *fig. 4B, C*), and at Stage III, when the ie is arising at the dorsal side (see *fig. 4D, E*).

Observations were made of two sections at both ends of three serial median or transmedian longisections of the ovule. Three apically situated cells per section were observed to judge whether they were dividing or had just divided periclinally. The frequency was obtained by means of the following formula..

$$\text{Frequency} = \frac{\text{Number of mother cells dividing or recently divided periclinally}}{\text{Number of cells observed}} \times 100$$

The result is summarized in *tables 1* and *2*. Although *E. equisetina* aas a whole shows a somewhat higher overall frequency than *E. distachya*, there is no significant difference in the basic pattern between the two species. As regards the oe and ie, the number of periclinal cell divisions in the dermal layer is remarkably high when they are swelling, the frequency attaining values of over 50% except at the dorsal side of the oe. In fact, the two-layered structure which has undoubtedly resulted from periclinal cell divisions in the original dermal layer becomes conspicuous at the stage when the oe is swelling, and the ie arises by periclinal cell divisions in the outer layer of the two-layered structure. In later stages the frequency of the oe decreases to less than 20% without exceptions. This means that the dermal cells tend to divide anticlinally or obliquely rather than periclin-

Table 1. The frequency of periclinally divided cells in the dermal layer of the oe and ie. (The number of cells observed for each calculation is put in brackets.)

		ventral side	lateral side	dorsal side
E. distachya	initial swelling	52%	56%	15%
oe		(81)	(48)	(123)
	early development	12%	13%	13%
		(90)	(108)	(108)
ie	initial swelling	58%	54%	52%
		(142)	(54)	(102)
E. equisetina	initial swelling	59%	67%	27%
oe		(51)	(24)	(60)
	early development	17%	14%	13%
		(84)	(36)	(105)
ie	initial swelling	74%	75%	67%
		(66)	(24)	(45)

ally. On the other hand, the topmost cells of the nucellus consistently show a higher frequency of periclinal cell divisions (from at least more than 50% up to nearly 90%).

4. DISCUSSION

The present study confirms some important characteristics of the reproductive histogenesis of *Ephedra* previously described. (1) In the early stages of ontogeny, two opposite projections of the oe arise at the lateral sides (JACCARD 1894), a fact indicating that the position of the two projections reflects a decussate phyllotaxy. STRASBURGER (1872, 1879) reported the two projections as lying at the lateral-ventral sides and not at the strictly opposite portion, and his description has been adopted by the later authors (PEARSON 1929; FAGERLIND 1971), but there can be no doubt that Strasburger observed stages too far advanced to permit the observation of sites of initiation of the two projections, since in both *E. distachya* and *E. equisetina* the two projections shift their position from the true lateral sides to the ventral side as the oe grows. (2) The oe is constituted by derivatives of the secondary dermal cells and the secondary subdermal ones (including some cells from the original subdermal layer) of the ovule primordium. PANKOW (1962) did not report the relation between the two-layered structure and the initial cells of the oe, but he described the oe to be of dual origin

Table 2. The frequency of periclinally divided cells in the dermal layer of the nucellus at the early stages of development.

	Stage I	Stage II	Stage III
E. distachya	53%	86%	57%
	(51)	(42)	(102)
E. equisetina	54%	89%	64%
	(24)	(36)	(75)

in *E. americana*. Probably in all species of *Ephedra* the oe is of dual origin, whereas the main part of the ie originates from the secondary dermal layer alone (STRASBURGER 1872; PANKOW 1962; FAGERLIND 1971).

As stated in the introductory remarks, many workers discussed the organographic nature and homology of the oe and the ie. However, most of them did not present any clear evidence whether they described both the oe and ie as having the same nature or a different one. FAGERLIND (1971) is probably the only author who adequately studied this aspect by ontogenetic observations. He partially documented the early ontogeny of the female reproductive organ (ovule) in several species of *Ephedra* (including *E. distachya*), and stated that there is no essential difference in this respect between the oe and ie, because their respective morphologies can be transformed from the one to the other, and he concluded that both the oe and ie are of a leafy nature. However, even Fagerlind's conclusion does not appear to be well founded, because he overlooked the significance of some important ontogenetic differences between the oe and ie.

The oe differs from the ie in the following respects: (1) the oe arises as a horseshoe-shaped primordium, whereas the ie arises as a ring-shaped one; (2) the oe has two projections at the lateral sides in early stage of its development, the ie, on the other hand, does not have such projections and shows an elevation at the ventral side during later stages; (3) the oe is vascularized in both its lateral sides (in the species investigated), but the ie does not have any vascular system; (4) the oe varies in thickness from part to part, but the ie is uniform except in the swollen basal part; (5) the oe does not form a micropylar tube, which is peculiar only to the ie; (6) the oe is of dual origin, but the main part of the ie is of dermal derivation. The above-mentioned differences in morphology and anatomy do not support the opinion that both envelopes are homologous. The fact that the oe, in marked contrast to the ie, but like the vegetative leaf (SEELIGER 1954), is formed by derivatives of both the dermal and subdermal cells may suggest that the oe is more comparable with the vegetative leaf than with the ie.

A few authors, such as STRASBURGER (1872), JACCARD (1894), PEARSON (1929), HAGERUP (1934), MEHRA (1950) and FOSTER & GIFFORD (1974), have suggested that the oe of *Ephedra* is a compound structure consisting of two laterally inserted leafy organs something like a pair of opposite bracts or bracteoles. As mentioned earlier, the present work confirms that the two projections arise oppositely at the lateral sides of the oe where two fused leafy organs may be expected. This fact, in conjunction with the histologically dual origin and the presence of vascular bundles, can be regarded as more reliable evidence for the above-mentioned opinion.

As regards the ie, I do not have any idea concerning the number and the arrangement of the constituent elements, although the ie exhibits a manifest elevation at the ventral side. A comment may be necessary on the basal swelling of the ie which progressively becomes more conspicuous in later stages. STRASBURGER (1872, 1879), THODAY & BERRIDGE (1912) and FAGERLIND (1971) noticed

the elongation between the nucellus-ie junction and the ie-oe junction, but they did not pay any attention to the growth in radial direction resulting from periclinal cell divisions. Probably this sort of bulging structure has not been recorded in other gymnosperms.

Most of the previous studies and the present one deal with species with two ovules per strobilus and with a decussate phyllotaxy. Species which have three ovules on the strobilus associated with trimery, and those which bear a solitary terminal (or pseudoterminal) ovule on the apex of the strobilus should be studied to generalize the characteristics of *Ephedra*, whereby the number and arrangement of the constituent elements of the oe and ie as well as their structural nature must be critically examined.

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