AN EMBRYOLOGICAL STUDY OF *LEVENHOOKIA DUBIA* SOND. IN LEHM.

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INTRODUCTION.

The genus *Levenhookia* R.Br. is restricted to South Australia and is placed in the second tribe Stylidieae of the family Stylidiaceae (Engler, 1908). The earlier literature on the embryological features of the family has already been reviewed by me elsewhere (1950).

MATERIAL AND METHODS.

L. dubia is a slender tiny erect herb growing to a height of one to two inches. The minute flowers are arranged in racemose clusters. The corolla is peculiar in having a grotesque labellum which encloses the column. The material used in this study was collected from a damp pasture land at Melbourne, South Australia, by Mr. J. H. Willis and Mr. O. D. Evans who very kindly passed it on to me for investigation. After following the customary methods for imbedding the material in paraffin, sections were cut at a thickness of 10 to 16 microns and stained in Heidenhain's iron alum-haematoxylin with eosin as a counterstain.

THE FLOWER.

Fig. 1 represents a longitudinal section of the flower to show the arrangement of the different floral parts. The flower is peculiar in having a column which originates from the apex of the inferior ovary and represents the fused style and stamens. The apex of the column ends in a bilobed stigma with one sessile anther on each side of it. The epidermal cells of the stigmatic lobes are papillate and densely cytoplasmic (Fig. 2).

At the base of the column and just above the inferior ovary is the nectary (Figs. 1, 3) composed of glandular cells with conspicuous nuclei and dense contents.

A number of glands are present on the pedicel of the flower, the outer wall of the inferior ovary and the sepals and petals. The mature gland (Fig. 4) consists of a biseriate stalk composed of a variable number of cells and bearing at its apex a group of four cells arranged in a sphere (Figs. 4, 5). The cells of the gland have conspicuous nuclei and dense cytoplasm.

The ovary is rounded to ovoid in outline, inferior and unilocular. The ovules are borne on a massive basal globose free central placenta (Figs. 1, 6). Those toward the upper portion of the placenta are anatropous and those below are hemitropous (Figs. 6, 7). This seems to be related to the breadth of the loculus which is greatest in the upper region but narrows toward the base. The wall of the ovary is made up of five to six layers of cells (Fig. 8). At the mature embryo-sac stage the cells of the innermost layer of the ovary wall become elongated and

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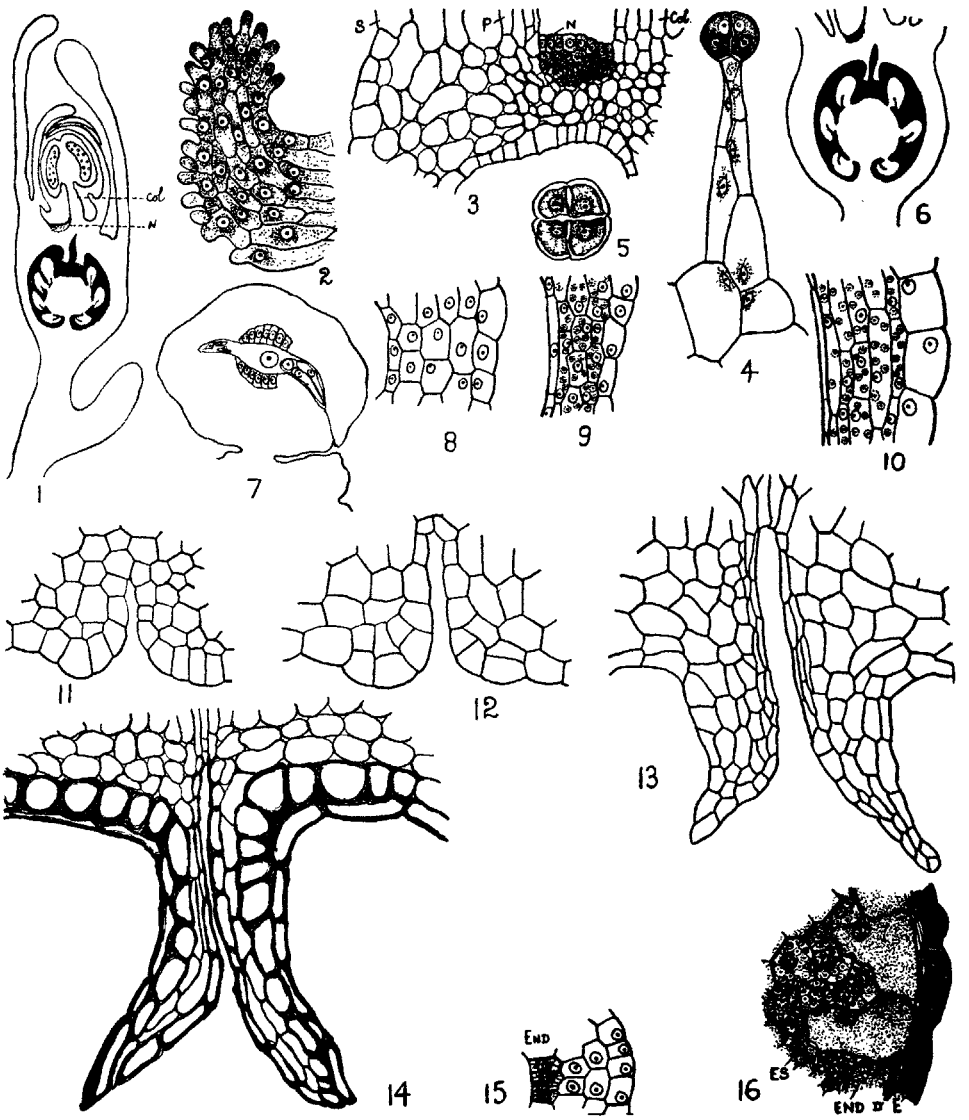


FIG. 1.—L.S. of flower to show arrangement of floral parts (*col.* = column; *n* = nectary). ×30.
FIG. 2.—A stigmatic lobe with papillose cells. ×215.
FIG. 3.—L.S. nectary (*col.* = column; *n* = nectary; *p* = petals; *s* = sepal). ×120.
FIG. 4.—A multicellular gland. ×215.
Fig. 5.—Top view of gland showing four cells. ×400.
FIG. 6.—L.S. ovary showing free central placentation. ×30.
FIG. 7.—A hemitropous ovule with embryo sac. ×215.
FIGS. 8–10.—Portions of ovary wall at various stages in development of fruit; in later stages the cells of the innermost layer become elongated and lignified. Fig. 8. ×291; Fig. 9. ×120; Fig. 10. ×120.
FIGS. 11–14. Stages in development of process arising from base of column and projecting downward into locule of ovary. Fig. 11. ×400; Fig. 12. ×485; Fig. 13. ×291; Fig. 14. ×120.
FIG. 15.—Portion of integument enlarged (*End.* = *Endothelium*). ×400.
FIG. 16.—Portion of seed coat enlarged (*E* = *epidermis*; *end.* = *endothelium*; *es.* = *endosperm*; *d* = crushed walls of the seed coat.) ×215.

thickened due to a deposition of lignin (Figs. 9, 10). At the same time the cells of the outer epidermis enlarge and a prominent cuticle is deposited over their outer surface.

An interesting feature seen in a longitudinal section of the ovary is the presence of a structure projecting downward from the base of the column and surrounding the apex of free central placenta (Figs. 1, 6). In very young stages the inner wall of the column bordering the apex of the loculus consists of a mass of homogenous cells with a narrow vertical slit in the centre (Fig. 11). A few of the epidermal cells lining the base of the slit undergo periclinal divisions (Fig. 12). By further divisions of these cells and the elongation of the hypodermal cells the projection becomes more pronounced. It presents a forked appearance in a longitudinal section and covers the apex of the free central placenta (Fig. 13). The forks slightly curve outwards and are composed of a mass of thin walled parenchymatous cells. Later, the epidermal cells become elongated and strongly lignified, and the cells of the subepidermal layer, which enlarge prominently, also become strongly thickened (Fig. 14).

A characteristic feature of this plant is the occurrence of starch grains in the ovary wall (Figs. 9, 10), the placenta, the cells of the integument and the endosperm (Figs. 16, 42–47).

Microsporangium and Male Gametophyte.

A transverse section through the young anther lobe shows a plate of four archesporial cells (Fig. 17), which divide periclinally to form the primary parietal and the primary sporogenous cells. By further periclinal divisions (Fig. 18) the primary parietal layer produces the anther wall and the sporogenous cells by undergoing a few more divisions become converted into the spore mother cells (Fig. 19). The epidermis is the outermost layer in the anther; next comes the endothecium; then a middle layer which soon becomes flattened and crushed; and finally the glandular tapetum whose cells are uninucleate at first (Fig. 19) but later become binucleate (Fig. 20). The microspore mother cells undergo the usual reduction divisions and form tetrads of microspores. Quadripartition of the microspore mother cells takes place by peripheral cleavage furrows (Fig. 21) and the microspores are arranged tetrahedrally.

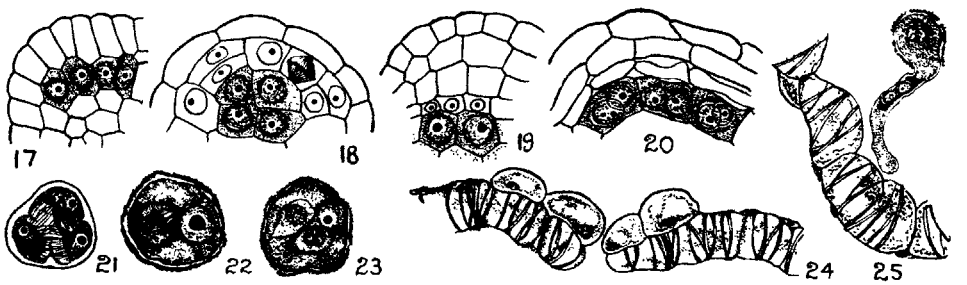


Fig. 17.—Portion of cross-section of anther showing archesporial cells. ×679.
Fig. 18.—Division of primary parietal layer. ×679.
Fig. 19.—Portion of young anther showing epidermis, endothecium, middle layer, uninucleate tapetum and sporogenous cells. ×679.
Fig. 20.—Same at later stage showing binucleate tapetum. ×679.
Fig. 21.—Quadripartition of microspores by formation of cleavage furrows. ×970.
Fig. 22.—Division of generative nucleus. ×970.
Fig. 23.—Mature pollen grain showing tube nucleus and male cells. ×970.
Fig. 24.—Portion of mature anther showing stomium, and fibrous endothecium. ×485.
Fig. 25.—Germination of a pollen grain *in situ*; note male cells in pollen tube. ×485.

In the mature anther the middle layer and the tapetum become disorganized and the endothecium develops the usual fibrous thickenings. The epidermal cells

also become shrivelled except those lying near the line of dehiscence which enlarge conspicuously (Fig. 24) and constitute the stomium.

The first division of the microspore results in the delimitation of a small lenticular generative cell from a large tube cell. The generative cell divides to produce two male cells (Figs. 22, 23).

In many anthers the pollen grains were found to have germinated *in situ*. Fig. 25 shows one such case with the two male cells in the tube. The tube nucleus is still in the pollen grain, and is showing signs of degeneration.

Megasporangium and Female Gametophyte.

The ovules appear as conical outgrowths on the placenta. The single integument appears just after the differentiation of the hypodermal archesporial cell and is made

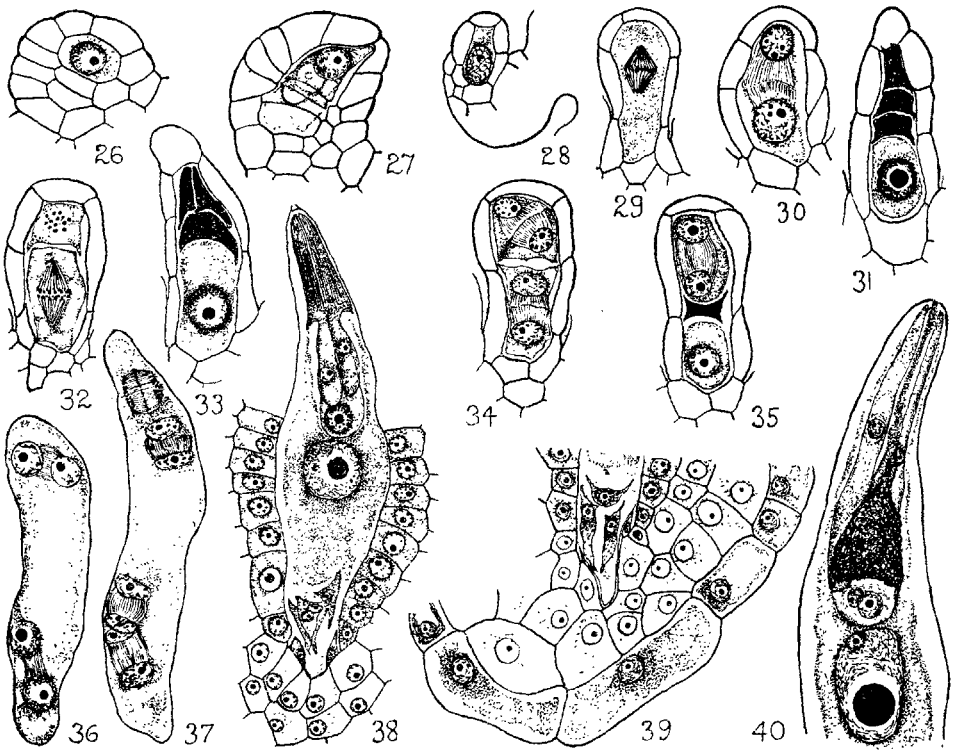


Fig. 26.—L.S. young nucellus showing primary archesporial cell. ×1000.
Fig. 27.—Three archesporial cells arranged in a superposed manner. ×800.
Fig. 28.—Megaspore mother cell. ×450.
Fig. 29.—Megaspore mother cell in division. ×679.
Fig. 30.—Dyad formation. ×1000.
Fig. 31.—Tetrad of megaspores. ×1000.
Figs. 32-33.—Stages in formation of T-shaped tetrad. Fig. 32. ×1000; Fig. 33. ×679.
Fig. 34.—Dyad cell dividing; note oblique division in upper dyad cell. ×1000.
Fig. 35.—Division in the lower dyad cell completed; upper dyad cell in telophase stage. ×1000.
Figs. 36-37.—Second and third nuclear divisions in embryo sac. ×1000 each.
Fig. 38.—Mature embryo sac showing elongated synergids and conspicuous endothelium; note filiform apparatus in the synergids. ×700.
Fig. 39.—Basal portion of embryo sac showing small chalazal process, pointed antipodal cells and endothelium. ×679.
Fig. 40.—A stage in double fertilization. ×970.

up of three layers of cells. Later, it becomes four-layered (Fig. 15) except in the region of the micropyle where the number of layers is usually smaller.

The primary archesporial cell (Fig. 26) directly functions as the megaspore mother cell (Fig. 28). Sometimes two to three archesporial cells (Fig. 27) are met with in the same nucellus (cf. *Stylidium garminifolium*, Subramanyam, 1950). As a rule tetrad formation takes place normally (Figs. 29, 30, 31). Sometimes the upper dyad cell divides in a plane at right angles to that of the lower (Figs. 32, 33), or the division is oblique (Fig. 34). Rarely it shows a belated division (Fig. 35). In any case, the chalazal megaspore functions. After undergoing three successive divisions (Figs. 36, 37) it produces an eight-nucleate embryo sac of the Polygonum type (Maheshwari, 1948).

During the further development of the embryo sac, the cells of the nucellar epidermis are completely destroyed, so that the embryo sac comes in contact with the innermost layer of the integument which becomes modified to form the endothelium (Figs. 15, 38). It consists of transversely elongated vacuolate cells with conspicuous nuclei.

The mature embryo sac (Fig. 38) tapers towards both ends. The synergids are longer than in most plants. They have a hooked apical end with a filiform apparatus, and a fairly broad basal end containing a large nucleus. The egg is pear-shaped and is situated between the synergids. The two polar nuclei fuse in the centre of the embryo sac to form a large secondary nucleus just prior to fertilization. The antipodals are organized into definite cells (Figs. 38, 39) and are usually pointed basally. They persist during the early stages of endosperm development (Figs. 41 to 47), but there is a tendency for the lower end of the embryo sac to grow beyond them as a very delicate process (Fig. 39) penetrating into the chalaza.

The pollen tube enters the embryo sac between the synergids (Fig. 40). Double fertilization takes place normally and immediately after the fertilization the synergids shrivel and degenerate.

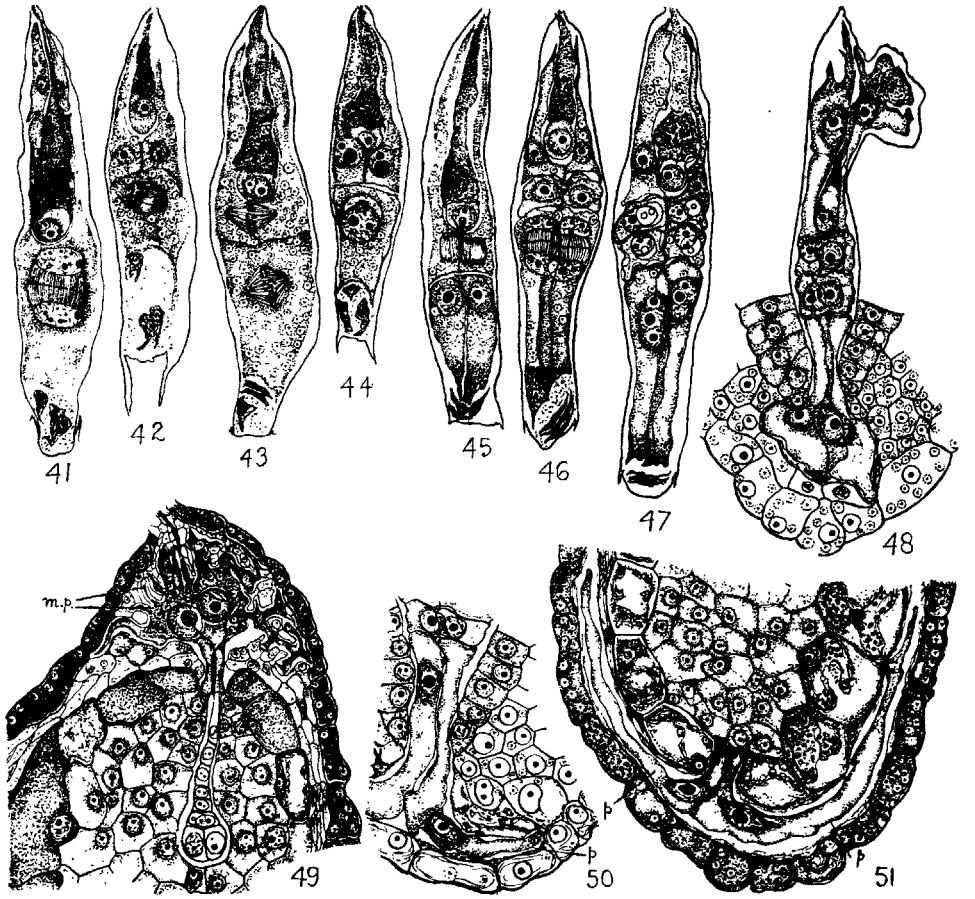
Endosperm.

The primary endosperm nucleus, which lies in the centre of the embryo sac, divides much earlier than the fertilized egg. The first wall is transverse (Fig. 41) followed by a vertical wall in the micropylar and then the chalazal chamber (Fig. 42). Or, the divisions in the two primary chambers are synchronous (Fig. 43). Sometimes the vertical wall in the chalazal chamber is laid down at right angles to that in the micropylar chamber (Fig. 44).

Transverse walls are next formed in each of these two tiers, first in the upper (Fig. 45) and then in the lower (Fig. 46). The result is an eight-celled endosperm made up of four tiers of two cells each.

At the eight-celled stage of the endosperm, the two cells of the upper tier form the micropylar haustorium, and those of the lower form the chalazal haustorium (Fig. 48). The two middle tiers by further divisions form the main body of the endosperm (Fig. 47), which later becomes packed with starch grains (Fig. 16).

Each cell of the micropylar haustorium contains a prominent nucleus embedded in a dense mass of cytoplasm (Fig. 48). It now becomes lodged in a fairly large cavity formed in the micropylar region of the integument by the enormous micropylar growth of the embryo sac. It forms a number of processes penetrating between the cells of the integument at the micropylar region (Fig. 49, *m*, *p*). At the same time the two cells of the chalazal haustorium disorganize the parenchymatous tissue in the chalazal region of the ovule and finally reach the epidermal layer of the seed, where they become distended (Fig. 48) and develop lateral prolongations or protrusions (Figs. 50, 51) which grow in between the cells of the integument.



FIGS. 41–47.—Stages in development of endosperm and differentiation of the micropylar and chalazal haustoria. All. ×679.
FIG. 48.—The young micropylar and chalazal haustoria respectively. ×485.
FIG. 49.—2-celled micropylar haustorium; processes (*m.p.*) from cells of the micropylar haustorium pass into the integument. ×291.
FIG. 50.—Chalazal haustorium at an early stage; note development of the prolongation (*p*) from a cell of the chalazal haustorium. ×485.
FIG. 51.—2-celled chalazal haustorium giving out the prolongations (*p*) which pass up into the integument. ×291.

SEED COAT.

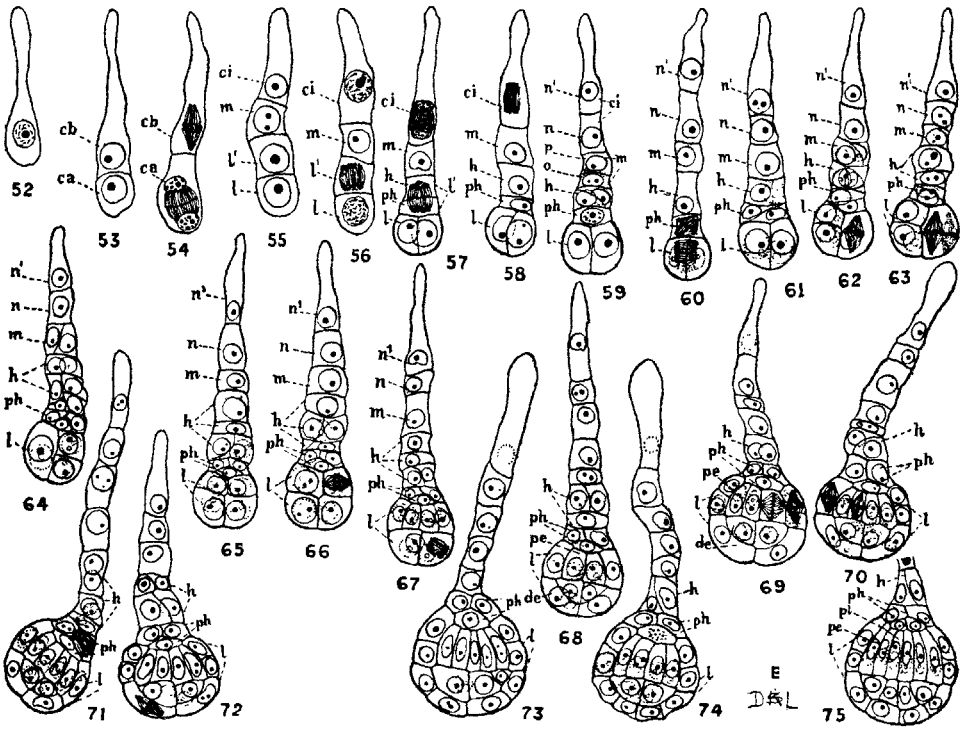
In the mature seed the cells of the outer epidermis and of the endothelium become filled with darkly staining material. Further, the cells of the endothelium enlarge prominently (Fig. 16) and assume an irregular outline. At the same time the middle layers of the integument are more or less completely crushed.

EMBRYO.

The fertilized egg elongates to form a long tubular structure (Fig. 52) and divides by a transverse wall to form two cells *ca* and *cb* (Fig. 53), both of which divide by transverse walls (Fig. 54) to form a filamentous proembryo of four cells *l*, *l'*, *m* and *ci*. Cells *l'* and *ci* divide transversely, either simultaneously (Fig. 57)

or in succession (Fig. 56), producing *ph*, *h*, *n* and *n'* respectively. The cell *l* now divides vertically (Fig. 57) and has the major part mainly in the formation of the embryo. The remaining cells above *l* except *ph*, by further divisions, constitute the filamentous suspensor.

The first division of *l* is followed by the laying down of another vertical wall at right angles to the former resulting in the quadrant stage (Figs. 58–61). The quadrants divide transversely resulting in the octant stage (Figs. 62–65). This is followed by further growth and cell divisions and the differentiation of the three histogens, dermatogen, periblem and plerome (Figs. 66–75).



FIGS. 52–75.—Stages in development of embryo (*ca* = terminal cell of the 2-celled embryo; *cb* = basal cell of 2-celled embryo; *m* and *ci* = cells derived from the basal cell *cb*; *l* = lower daughter cell of *ca*; *l'* = upper daughter cell of *ca*; *ph* and *h* = daughter cells of *l'*; *o* and *p* = daughter cells derived from *m*; *n* and *n'* = daughter cells derived from *ci*; *de* = dermatogen; *pe* = periblem; *pl* = plerome). Figs. 52 to 68. ×500 each; Figs. 69 to 75. ×679 each.

At about the time of the first vertical division in the terminal cell of the proembryo, *h* divides obliquely (Fig. 59) or vertically (Fig. 61) forming two cells which undergo transverse and vertical divisions so as to produce a small group of cells (Figs. 62–66). The cell *m* also sometimes divides producing two cells (Figs. 59, 62, 64). As a result of these divisions, the middle portion of the suspensor presents a biseriate appearance (Figs. 62, 64).

At about the quadrant stage of the embryo, *ph* (Fig. 60) divides by an oblique wall (Figs. 60–63) followed by another intersecting oblique wall at the octant stage (Figs. 64, 65) resulting in the formation of a three-celled hypophysis (Figs. 66–73). The outer two cells complete the dermatogen and get more or less merged in this

layer (Figs. 72–75), while the inner cell becomes embedded in the upper portion of the embryo (Figs. 69–73) where it divides vertically (Figs. 74-75) and becomes a part of the plerome.

Discussion.

As is characteristic of Stylidiaceae, the flower of *Levenhookia* has a column with a nectary at its base. In *Stylidium graminifolium* (Subramanyam, 1950) two prominent nectaries are present at the base of the column. Stalked multicellular glands are present on the parts of the flower in both genera.

The ovary is unilocular with a number of ovules borne on a free central placenta. The ovules in the upper region of the placenta are anatropous but those below are hemitropous. This appears to bear some relation to the breadth of the locule which is greatest in the upper region but narrows towards the base. The tendency towards a unilocular condition in the members of this family is very significant since it points the way towards the unilocular ovary of the Compositae. Another interesting feature noticed in a longitudinal section of the ovary of *Levenhookia dubia* is the presence of a rimmed projection hanging from the base of the column into the locule of the ovary.

The development and structure of the microsporangium of *Levenhookia* closely resembles that of *Stylidium*. In many anthers of the former the pollen grains were found to have germinated *in situ* as in *S. graminifolium* (Subramanyam, 1950) and in some members of the closely allied family Lobeliaceae (Kausik and Subramanyam, 1945; Subramanyam, 1949).

The development of the embryo sac conforms to the Polygonum type. The antipodals persist during the early stages of endosperm development. The lower end of the embryo sac grows past them as a very delicate process, penetrating into the chalaza as in *S. graminifolium* (Subramanyam, 1950).

The endosperm is cellular and the course of divisions closely resembles that in *Stylidium* (Rosén, 1935, 1949; Subramanyam, 1950). At the eight-celled stage of the endosperm, the two upper and the two basal cells develop into the micropylar and chalazal haustoria respectively. In later stages the micropylar haustorium gives out lateral processes which grow in between the parenchymatous cells of the integument as in *S. graminifolium* (Subramanyam, 1950). Such a tendency has also been seen in certain members of Orobanchaceae (Crété, 1942; Tiagi, 1950) and Plantaginaceae (Crété, 1942). The two-celled chalazal haustorium develops long tubular prolongations which grow in between the cells of the integument. In *S. graminifolium* (Subramanyam, 1950) the chalazal haustorium is made up of two uninucleate cells and both form a number of processes which grow in between the cells of the integument. In *S. adnatum* and *S. graminifolium* (Rosén, 1935, 1949), however, the chalazal haustorium is not so well developed.

The development of the embryo corresponds broadly to the Solanad type of Johansen (1945). The penultimate cell forms the hypophysis. In *S. graminifolium* (Subramanyam, 1950) the four terminal cells of the filamentous proembryo take part in the formation of the different regions of the embryo.

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Summary.

The flower has a column formed from the fused style and stamens. A structure projects vertically from the base of the column into the locule of the ovary and becomes thickened in the seed. Multicellular stalked glands of epidermal origin are present on the pedicel and on the surface of the floral parts excepting the column.

The wall of the anther consists of three layers of cells external to the binucleate tapetum. The endothecium shows the usual fibrous thickenings. A stomium is present. The pollen grains are three-celled and frequently germinate *in situ*.

The ovary is inferior and unilocular with a number of unitegmic ovules borne on a basal globose free central placenta. The ovules at the top are anatropous but those at the base are hemitropous. The innermost layer of the integument forms a prominent endothelium. During post-fertilization stages the cells of the innermost layer of the ovary wall become elongated and lignified.

There is usually a single archesporial cell which functions directly as the megaspore mother cell. Occasionally two or three archesporial cells are found. Megasporogenesis proceeds normally and the embryo sac is of the Polygonum type. The synergids are elongated, and show a filiform apparatus and the characteristic hook-like projections. The antipodal cells are pointed at their lower ends and the embryo sac forms a small process which passes beyond them into the chalaza. Double fertilization has been observed.

The endosperm is cellular and follows the Scutellaria type of Schnarf (1931). The micropylar haustorium is two-celled and forms a number of processes penetrating between the cells of the integument at the micropylar region. The two-celled chalazal haustorium develops long tubular prolongations which reach down to the outer epidermis of the seed coat and then grow up in between the cells of the integument.

The development of the embryo has been described in detail. Broadly, it follows the Solanad type of Johansen (1945). The terminal cell of the filamentous proembryo gives rise to the main body of the embryo. The penultimate cell is the hypophysis.

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