Prof. T.Pullaiah,

Professor of Botany,

Sri Krishnadevaraya University,

Anantapur 515003, A.P.

The Journal of the Indian Botanical Society

(Formerly " The Journal of Indian Botany ")

VOL. XXIV] MAY, 1945 [No. 2

EMBRYOLOGICAL STUDIES IN THE THYMELÆACEÆ

I. *Thymelaea arvensis* Lamk.

BY J. VENKATESWARLU

Andhra University

Received for publication on February 10, 1945

THYMELÆACEÆ, by itself a very natural group, shows no close affinities with other families of flowering plants. It has been investigated by embryologists from time to time, but these investigations are mainly concerned with the development of pollen, ovule and embryo-sac. A few observations have been made also on the development of the embryo, but these accounts are very fragmentary and do not give either a correct or a complete picture of embryo development in any species. This has led the author to take up the present work.

PREVIOUS WORK

Broniart (1826) was the earliest botanist to pay attention to the embryology of Thymelæaceæ. He recognised the conducting function of the tissue arising at the base of the style (now called the obturator). Next, Hofmeister (1849) studied *Daphne laureola*, in which he observed a normal type of embryo-sac development. He also recorded that the mature embryo-sac was already formed before the onset of winter. Beauregard (1877) investigated the structure and development of the fruit in *Daphne*. Capus (1878), in his studies on the anatomy of conducting tissue in the angiosperms, drew attention to the formation of a bundle of tissue traversing from the base of the style to the micropyle. Vesque (1879) made some observations on *Daphne mezereum*. According to him, the archesporium in the ovule consists of a single cell and the embryo-sac development follows the normal type. The formation of nucellar cap and obturator was also studied by him. Prohaska (1883) studied the development of the embryo-sac and the endosperm in *Daphne*. He reported the occurrence of many antipodals in *Daphne Blagayana*. Schimper (1885) is referred to by Schnarf (1929)

as having reported the occurrence of plastids in the egg of the above species.

Strasburger (1884, 1885, 1909 and 1910) studied the embryology of *Daphne*, *Gnidia* and *Wikstræmia* and particularly the problem of parthenogenesis in *Wikstræmia indica*. He noted unicellular archesporium in the ovule, a normal type of embryo-sac development, many antipodals in the embryo-sac of *Daphne Blagayana* and only three in *Daphne alpina* and *Wikstræmia indica*, and the formation of the nucellar cap in the ovule. The development of the obturator and the endosperm in *Daphne* was also studied by him. He also made some observations on the structure and development of the anther and pollen. He states that the tapetal nuclei divide mitotically in *Wikstræmia indica*.

Winkler (1904, 1906) made an embryological study of *Wikstræmia indica* and investigated the problem of parthenogenesis in it. He traced the structure and development of the anther and pollen. According to him, the tapetum is formed by the innermost wall layer of the anther and its cells contained 2–6 nuclei. He described a normal structure in the embryo-sac. He studied the formation of obturator and pointed out that it formed a plug in the micropyle in *Wikstræmia indica*. He also made some observations on the development of the endosperm and embryo.

Osawa (1913) investigated the development of pollen and embryo-sac of *Daphne* with special reference to sterility in *D. odora*. According to him, the division in the pollenmother cell is simultaneous. He reported the 3-nucleate condition of the mature pollen, and also some irregularities in the pollen formation in *D. odora*, besides degenerations in pollen and embryo-sacs. According to him, a linear tetrad of megaspores is usually formed in *D. odora*, but he also observed occasional formation of a T-shaped tetrad. Normally the chalazal megaspore is the functional one. He, however, found a few exceptional cases in *D. odora*, where a megaspore other than the chalazal one of the tetrad was functional. He noted 3–6 antipodals in the embryo-sac in *D. odora* and 30 or more in *D. pseudo-mezereum* and *D. koiusiana*.

Guèrin (1913, 1915) made a comprehensive study of the structure and development of the ovule and seed in Thymelæaceæ. He found vascular strands in the nucellus of *Dicranolepis*, *Craterosiphon* and *Synaptolepis*. His observations on the structure and development of the embryo-sac agree with those made earlier by others. He reported the occurrence of two cases of bilateral tetrads of megaspores in *Daphnopsis Schwartzii*. Guérin also reported more than three antipodals in *D. Schwartzii* and many in *Thymelæa passerina* and *Dirca palustris*. He made some observations on the endosperm. Structure of the seed-coat was also described.

Dahlgren (1915) in his studies on the development of pollen, ovule and seed in angiosperms, mentions the formation of normal type of embryo-sac in *D. mezereum* before the advent of winter. Yamaha

(1926), in his comprehensive study of cytokinesis in the formation of pollen tetrads in the various plant groups, states that the cytokinesis in *Daphne* takes place by furrowing and passes through very rapidly. He also refers to the formation of a transitory cell plate after the heterotypic division in the pollen mother cells. Joshi (1937) noted 3-nucleate condition in the mature pollen of *Wikstræmia indica*, *Thymelæa arvensis* and *Daphne mezereum*.

Fuchs (1938) gave a detailed account of the embryology of *Daphne odora* and made some observations on the embryology of *D. cneorum* and *Passerina pectinata*. She made a comparative study of the structure of pollen in *Daphne odora*, *D. cneorum*, *D. Blagayana*, *D. mezereum*, *Passernia pectinata*, *P. filiformis*, *Pimelea decussata*, *Pimelea ligustrina* and *P. spectabilis*. In all these she finds the mature pollen to be 3-nucleate and sperm cells to be elongated in the form of spirally twisted bands. She also describes the presence of rods and spines as structural features of the exine. The pollen has many germ pores. In *D. laureola*, *D. cneorum* and *Passerina pectinata*, she notes the formation of a nucellar cap, the presence of a plate-shaped tissue in the chalaza of the ovule and a conducting strand of elongated cells connecting it with the antipodal end of the embryo-sac. She also gives an account of the structure of the obturator. The embryo-sac development in the species studied follows the normal type. She reports the occurrence of linear, T-shaped and bilateral tetrads of megaspores. She finds 30–40 antipodals in the embryo-sac of *Daphne laureola*, *D. cneorum* and *Passerina pectinata*. According to her, these persist as dark points in ripe seeds. She states that polar nuclei lie close together near about the chalazal end and probably fuse just before fertilisation. The same was observed earlier by Winkler (1906), Strasburger (1909) and Guérin (1915). She followed the endosperm development in *D. laureola*. The fusion nucleus, according to her, divides before the fertilised egg. In the later stages cell formation was also noted by her. She also made a few observations on the embryo development in *D. laureola* and described the seed-coat structure.

Mauritzon (1939) described the structure and development of the ovule and seed in *Phaleria capitata*. He observed a remarkably extensive growth of the chalazal part in the ovule. He also made a few observations on the development of the pollen and embryo-sac in the same species.

Kausik (1940) published an account of the structure and development of the embryo-sac in *Lasiosiphon eriocephalus*. He described the structure of the anther and traced the origin of the tapetum. He noted the formation of a nucellar cap and conducting strand in the nucellus below the antipodal end of the embryo-sac. The development of the embryo-sac is normal. He noted the occasional presence of a spherical body in the synergids. According to him, the endosperm is of the free nuclear type. In the early stages the endosperm nuclei show a paired arrangement. He described a dense mass of cytoplasm in the chalazal part of the embryo-sac and states that the endosperm nuclei are large in this region and lie embedded in the plasma accumulated there.

He also made a few observations on the embryo. A 3-celled proembryo is formed. Further development is not followed in detail, but he states that a spherical embryo is formed after a few divisions. He also described the seed-coat structure, which is in agreement with the earlier accounts.

Material and Methods

The present paper deals with the development of the anther, pollen, ovule, embryo-sac, endosperm, embryo and seed in *Thymelæa arvensis* Lamk. This species grows in the upper Punjab, Kashmir, N.W.F.P., and extends from Afghanistan westwards to France and North Africa.

The material used in this investigation was very kindly placed at my disposal by Dr. A. C. Joshi of the Benares Hindu University along with a few prepared slides. It was collected from plants growing at Sopore (Kashmir), in the month of June 1938 and was fixed in formalin-acetic-alcohol. The customary methods of dehydration and infiltration were followed. Sections were cut 10–14 μ in thickness and were stained with Heidenhain's iron-alum-hæmatoxylin. Tuan's method of destaining with picric acid was followed.

Organogeny of the Flower

The flowers arise singly in the axil of leaves. The floral parts arise in acropetal succession, the perianth making its appearance first, followed by the stamens and, last of all, the gynœcium (Figs. 14 *a*, *b* and *c*). The single whorl of perianth is urceolate and its 4 lobes are imbricate in bud. The upper part of the perianth shrivels up in the fruiting stage and the whole perianth remains as a membranous covering round the ovoid fruit.

The stamens are 8 in number (in two whorls) and are adnate to the perianth. The ovary is raised on a short stalk and contains a laterally attached single ovule. The style is short and ends in a stigma the surface cells of which form a number of papillæ. A prominent annular disc-scale develops round the base of the ovary.

Development of the Anther and Pollen

The primary archesporium in the anther consists of only one row of cells in each of the four anther-lobes (Figs. 1 and 2). Soon after their differentiation the primary archesporial cells undergo a periclinal division, forming a layer of primary parietal cells towards the outside and a layer of primary sporogenous cells towards the inside (Fig. 3). The parietal layer of cells again undergoes another periclinal division and forms two layers of wall cells below the epidermis (Fig. 4). The inner of these only divides further into two layers, thus ultimately forming three wall layers below the epidermis (Fig. 5). The layer of wall cells immediately beneath the epidermis forms the endothecium, while the one immediately outside the sporogenous cells forms the tapetum. It is of the secretory type.

The tapetal nuclei divide mitotically and the tapetal cells become two-nucleate about the time when the pollen mother cell nuclei are in

the prophase of I meiotic division. The two nuclei lie closely appressed to each other. In the final stages the tapetal cell nuclei are found to contain up to 4 nucleoli. The tapetal cells, before degeneration, become filled with very small vacuoles.

In the mature anther, due to the growth of the pollen mother cells and the tapetum, the wall layer between the endothecium and the tapetum gets crushed. With the further growth of the anther, the epidermis gets very much stretched, the endothecium further enlarges and the tapetum slowly degenerates. In very mature anthers the epidermis is so thinned out that it is hardly perceptible as a separate layer at some places (Fig. 6).

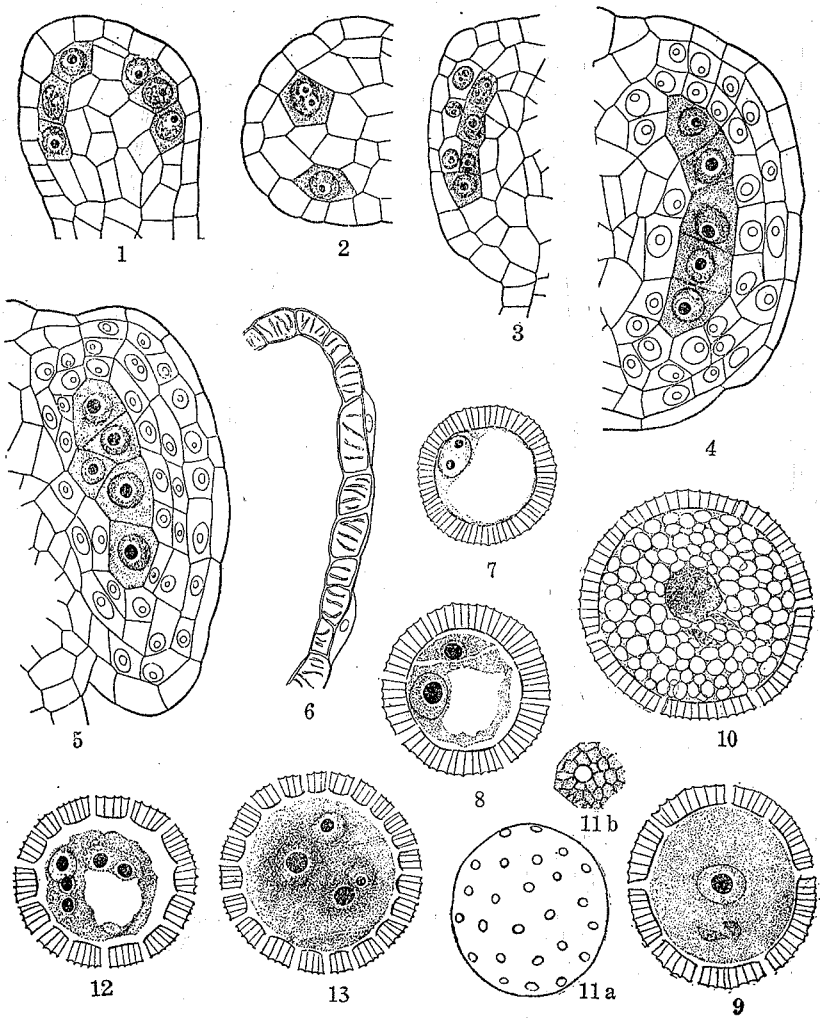
Usually the primary sporogenous cells enlarge and form the pollen mother cells. However, quite frequently, a cell or two of the primary sporogenous layer may divide once before forming pollen mother cells. The pollen mother cell nuclei undergo two meiotic divisions and form pollen tetrads ultimately. During meiosis they do not round off but remain packed together within the mother cell walls. The pollen mother cells divide simultaneously. The orientation of spindles at the II meiotic division has been found to be at right angles to each other, but sometimes parallel arrangement is also seen. This results in the formation of both tetrahedral as well as bilateral pollen tetrads. Cytokinesis appears to pass through rapidly and takes place by furrowing.

The exine and intine are formed before the pollen grain becomes two-nucleate. Fully formed uni-nucleate pollen grain has a large vacuole and the nucleus occupies a peripheral position. Usually, it is 1-nucleolate, but two nucleoli have been observed quite frequently (Fig. 7). The nucleus divides and forms two nuclei which show considerable difference in their size. The larger one is the vegetative nucleus and the smaller is the generative nucleus. The latter is organised into a definite lenticular cell separated from the vegetative one by a curved wall (Fig. 8). This wall, however, soon disappears, both the nuclei being left in the general cytoplasm of the pollen grain. The formation of such a lenticular cell separated from the vegetative one by an evanescent wall was also noted in some Thymelæaceæ by Fuchs (1938).

The generative nucleus divides into two male nuclei which finally form two elongated sperms. The pollen grain thus becomes 3-nucleate, which condition has been noted as characteristic feature of the pollen in Thymelæaceæ (Osawa, 1913 ; Joshi, 1937 ; Fuchs, 1938). The pollen grain shows a general increase in size and in mature condition gets filled with starch grains (Fig. 10). The sperms are elongated and spindle-shaped. The chromatin is unevenly distributed and the sperms look like spirally twisted bands. The same was also observed by Fuchs (1938) in *Daphne*. The tube nucleus is spherical in the early stages, but later it takes an irregular shape and stains very deeply as in Amarantaceæ (Kajale, 1940).

The exine and intine are formed in the uni-nucleate stage of the pollen grain. The former is much thicker than the intine. As seen

in sections, it is composed of light and dark staining parts, the latter taking the form of radially arranged rods covered on all sides by the light staining portions of the exine (Figs. 7–10, 12 and 13). The exine protrudes a little outwards on the surface where the rod-like portions are situated giving rise to small spines. The latter are united



Figs. 1–13. *Thymelæa arvensis*.—Figs. 1-5. Various stages in the development of the anther. Fig. 2. Shows a transverse section, the rest represent longitudinal sections. Fig. 6. L.S. of wall of a mature anther. Figs. 7–10. Pollen grains in various stages of development. Fig. 11 *a*. Surface view of pollen grain showing germ pores. Fig. 11 *b*. A portion of exine showing a germ pore and sculpture on the surface. Figs. 12 and 13. Abnormal pollen grains showing extra nuclei. Figs. 1-5, ×546; Fig. 6, ×263; Figs. 7–11 *a* and 12–13, ×833; Fig. 11 *b*, ×3,360.

on the surface by ridges giving rise to a network like pattern on the outer surface of the exine (Fig. 11 *b*). The meshes of the network are usually 6-sided. There are many germ pores (about 50) and they are approximately equally spaced. They are usually circular in outline. Their arrangement on the exine is rather interesting. Usually, each pore, taken as centre, is surrounded by six other pores (Fig. 11 *a*). The pore membrane does not protrude out of the germ pores.

The mature pollen is sphæroidal in shape and the exine gets thinner on account of the stretching of the wall due to the growth of the protoplast of the pollen grain, very much like what has been described by Kajale (1940) in some Amarantaceæ. The diameter of the mature pollen grain approximately measures 35 μ–37 μ and that of the germ pore 1·6 μ–1·8 μ. The distance between two germ pores (centre to centre) is about 5–6 μ.

Abnormal Pollen

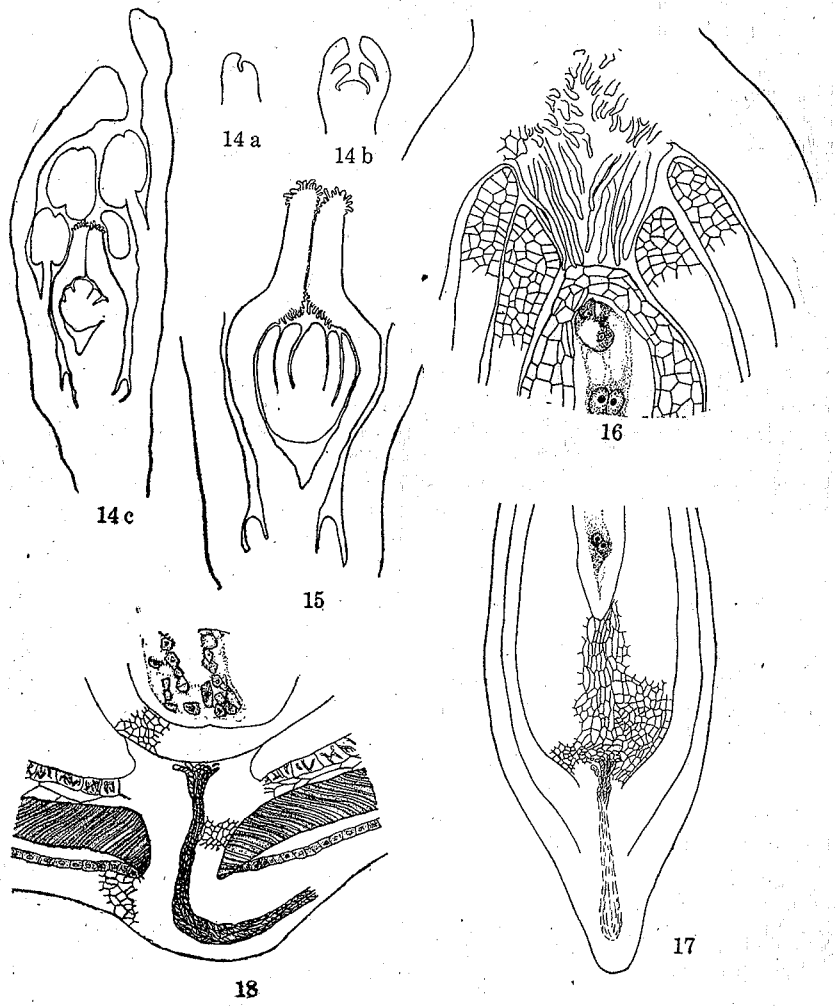
The pollen grains, as stated above, are usually 3-nucleate at the shedding stage. When the generative nucleus divides, it forms two sperm nuclei which are round in shape in the beginning. The vegetative nucleus is bigger in size and possesses a nucleolus. It stains less deeply than the generative nucleus with iron-alum-hæmatoxylin. The latter is distinguished from the vegetative nucleus by the absence of a distinct nucleolus, smaller size and deep staining capacity with iron-alum-hæmatoxylin. These features are also shared by the two sperm nuclei that arise from it.

Two exceptional cases of multi-nucleate pollen have been encountered in my preparations (Figs. 12 and 13). Fig. 12 shows a pollen grain with 5 nuclei, out of which one is larger in size. It shows a distinct nucleolus and stains less deeply than the rest with iron-alum-hæmatoxylin. The rest of the four are much smaller in size, take a deep stain and show no distinct nucleolus. From these features, it appears that the two sperm nuclei have undergone an extra division producing four daughter nuclei resembling the sperm nuclei in their general appearance. It may be mentioned here that four male nuclei (of about equal size), in pairs, have been observed by Dutt and Subba Rao (1933) in some pollen grains and in a pollen tube that has just reached the embryo-sac in the sugarcane (cross vellai ♀ × B. 3412 ♂). Fig. 13 shows a pollen grain with four nuclei, out of which two are larger in size, stain less deeply and show a nucleolus, while the rest of the two resemble sperm nuclei. In this case, it appears that the extra nucleus has arisen due to the division of the vegetative nucleus.

Wulff and Maheshwari (1938) list a number of such abnormal cases of pollen having more than three nuclei in their review of the male gametophyte of angiosperms. The list includes *Lilium tigrinum* (Chamberlain, 1897), *Eicchornia crassipes* (Smith, 1898), *Sparganium simplex* (Campbell, 1899), *Yucca recurva* (Woycicki, 1911), *Cuscuta epithymum* (Federtschuk, 1931), *Atriplex hymenolytra* (Billings, 1934) and *Stelleria media* (Joshi, P. C., 1936). Bhargava (1936) reports

a 4-nucleate condition in many pollen grains of *Chenopodium album*. Juliano and Alcala (1935) reported the occurrence of 2–7 vegetative nuclei in *Musa errans* (Blance) Teodore var. *Botoan* Teodore.

Citing some of the above cases, Coulter and Chamberlain (1903) write, "In *L. tigrinum*, Chamberlain has often found a small cell cut



Figs. 14–18. *Thymelæa arvensis*.—Figs. 14 *a*, *b*, *c*. Stages in the development of the flower. Fig. 15. L.S. of the gynœcium showing the development of the obturator, ovule and the disc scale at the base of the stalked ovary. Fig. 16. L.S. base of the style and upper part of a mature ovule showing the obturator in the funnel-shaped micropyle. Fig. 17. L.S. of ovule (except the micropylar part) showing the conducting strand connecting the antipodal end of the embryo-sac with the ovular vascular trace. Fig. 18. L.S. of the chalazal end of an old ovule. Figs. 14 *a*, *b* and *c*, ×185; Fig. 15, ×78; Fig. 16, ×213; Figs. 17 and 18, ×126.

off by the microspore before the appearance of the tube and generative nuclei and the same cell was noted after the division of the generative nucleus. A similar cell was found by Smith in *Eicchornia crassipes* and by Campbell in *Sparganium simplex*. It is suggestive of true vegetative or prothallial cell, two of which so commonly occur in gymnosperms but the phenomenon is too unique as yet among the angiosperms to deserve more than a mention." According to Wulff and Maheshwari, Billings (1934) does not think it improbable that "an angiosperm would now and then be found exhibiting an atavistic tendency in producing a prothallial cell". P. C. Joshi (1936), however, thinks that there is no scope for interpreting the extra nucleus as a prothallial cell in the exceptional case of the 4-nucleate pollen grain recorded by him in *Stelleria media*. From the two cases recorded by the writer, it appears that the extra nuclei may be formed due to the further division of either the vegetative nucleus or the generative one.

DEVELOPMENT AND STRUCTURE OF THE OVULE AND EMBRYO-SAC

Ovule.—The ovary contains a single anatropous ovule. It is laterally attached, with the micropyle pointing upwards. First, it appears as a small hump. At about the time of division of the primary archesporial cell into an outer parietal cell and an inner megaspore mother cell, the integuments start as two annular rings one below the other. Ultimately the fully developed ovule assumes the anatropous form. In the early stages of development there is some space below the ovule (Figs. 14*c* and 15), but afterwards it is all occupied and the ovule touches the lower end of the ovary. There is no suggestion of the presence of a second ovule.

The ovule has two integuments. The micropyle, in fertilisable ovules, is funnel-shaped. It is formed by both the integuments, though the inner takes greater part at this stage. The micropyle receives the obturator. After fertilisation, the obturator dwindles gradually until, finally, it disappears in the seed. The integuments also grow and come close together forming a narrow micropyle in the seed. To start with the integuments are 3 cells in thickness, but in later stages the outer one becomes 4 cells in thickness and the inner 5 cells in thickness. Their various cell layers undergo different changes in the seed-coat. These are described later.

The nucellus is fairly massive. The nucellar epidermis, in the micropylar region, shows periclinal divisions and forms a nucellar cap 2–3 cells in thickness (Figs. 21, 22 and 23). In the mature ovules, the nucellus above the micropylar part of the embryo-sac is about 4–5 cells in thickness (including the cells of the nucellar cap) and, at the apex forms a slightly elongated protrusion. Usually it is about 4 cells in thickness on the sides of the embryo-sac and 15–20 cells in thickness below the antipodal end of the embryo-sac. In the chalazal region is developed a strand of elongated cells connecting the vascular bundle of the funicle and the antipodal end of the embryo-sac (Fig. 17). Such a conducting strand in the chalazal region of the ovule was also noted by Fuchs (1938) in some members of Thymelæaceæ and by

Kausik (1940) in *Lasiosiphon eriocephalus*. Guérin (1913) described the presence of vessels in the nucellus of *Dicranolepis*, *Craterosiphon* and *Synaptolepis*. Such vessels, however, are absent in *Thymelæa arvensis*, which, in this respect, resembles *D. laureola*, *D. cneorum*, *Passerina pectinata* (Fuchs, 1938) and *Lasiosiphon eriocephalus* (Kausik, 1940). A similar conducting strand in the ovule is also found in Lythraceæ (Joshi and Venkateswarlu, 1935 *a*, 1935 *b*, 1936; Venkateswarlu, 1937 *a*), in *Duabanga sonneratioides* (Venkateswarlu, 1937 *b*) and in Geissolomataceæ (Stephens, 1909).

Megasporogenesis and Embryo-sac.—The primary archesporium in the ovule consists of a single cell, which differentiates much before the integumental primordia appear (Fig. 19). It undergoes a periclinal division giving rise to an outer cover cell and an inner megaspore mother cell. Even before this division, the cells of the epidermis have divided once or twice periclinally to form the nucellar cap. The primary parietal cell undergoes a periclinal division and ultimately gives rise to two layers of parietal tissue under the nucellar cap. In *Daphne alpina* (Strasburger, 1909), *D. mezereum* (Vesque, 1879), *D. odora* (Osawa, 1913), *D. laureola*, *D. cneorum* and *Passernia pectinata* (Fuchs, 1938), a more extensive parietal tissue is formed making the megaspore mother cell deep-seated.

The megaspore mother cell forms a linear tetrad of megaspores, the chalazal-most of which is the functional one (Figs. 21–22). The three micropylar megaspores degenerate and they can be seen at the 4-nucleate stage of the embryo-sac (Fig. 23). No cases of T-shaped or bilateral tetrads of megaspores have been met with as in *D. alpina* (Strasburger, 1909), *D. odora* (Osawa, 1913), *D. Schwartzii* (Guérin, 1915) and *D. laureola* (Fuchs, 1938).

The development of the embryo-sac is according to the normal type (Figs. 21–27). The binucleate embryo-sac is characterised by the persistence of the chalazal vacuole. The same feature has been observed in *Lasiosiphon eriocephalus* (Kausik, 1940) and in Lythraceæ (Joshi and Venkateswarlu, 1935 *a*, 1935 *b*, 1936). In the 8-nucleate embryo-sac, the organization of the antipodals takes place slightly earlier than the egg-apparatus (Fig. 24). The two polar nuclei move towards the centre and meet about the middle of the embryo-sac. Usually the polar nuclei are 1-nucleolate, but in one case each of them has been found to be 2-nucleolate (Fig. 25). They fuse just before triple fusion. The two synergids, when fully developed, are hooked and have the usual chalazal vacuole. There is also seen a small vacuole in the apical region of the synergid (Fig. 28). The egg is usually situated a bit deeper than the synergids (Fig. 27) and has the usual flask-shaped form with a large vacuole above the nucleus (Fig. 29). In the early stages of the 8-nucleate embryo-sac, the antipodals are formed into three cells. Later, they multiply and form a large number of small cells (Figs. 27 and 31). Usually about 25–30 cells may be counted. During the endosperm formation, the antipodal end of the embryo-sac elongates and leaves behind the antipodal mass of cells on one side at about the middle of the embryo-sac (Figs. 32 and 33).

As can be seen from the review of previous work, many antipodals have been reported previously in *Daphne Blagayana*, *D. pseudo-mezereum*, *D. koiusiana*, *D. laureola*, *D. cneorum*, *Passerina pectinata*, *Dirca palustris* and *Thymelæa passerina*.

The form of the embryo-sac varies at the various stages of its development (Figs. 24–29 and 30–34). Up to the time of fertilisation it is contained in the upper half of the nucellus, but later it elongates and extends throughout the length of the nucellus. With the growth of the embryo-sac the surrounding nucellar tissue is crushed.

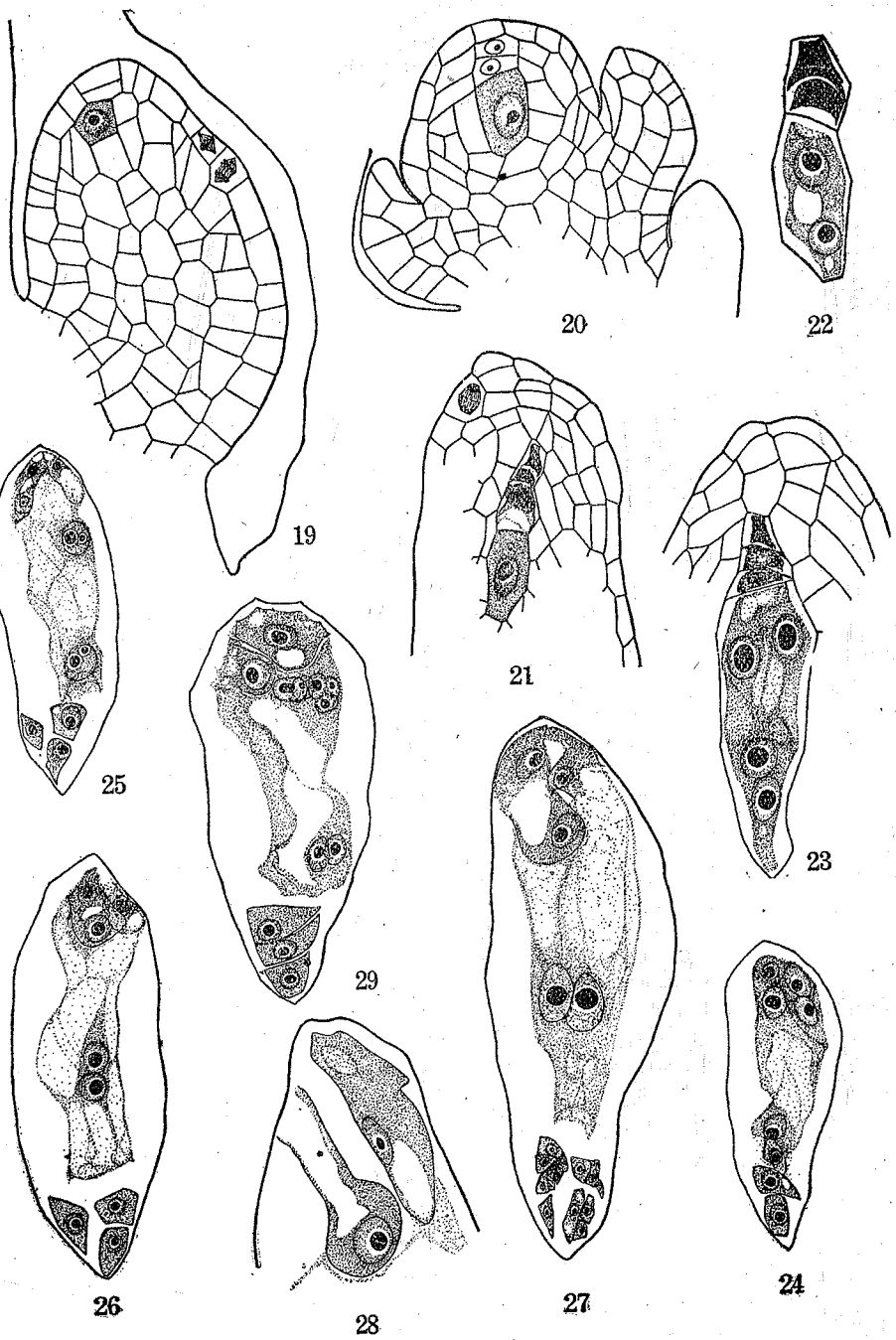
An Abnormal Embryo-sac

As stated above, the embryo-sac in *Thymelæa arvensis* is formed according to the normal type. The mature embryo-sac contains very small antipodals in addition to the egg apparatus and the two polar nuclei. A single case of an abnormal embryo-sac with more than the usual number of nuclei has been met with. Fig. 29 shows the same. It is in an early stage of development and shows three antipodal cells and two polar nuclei lying together near the antipodal end, while in the micropylar end are seen two cells and five nuclei. Out of the two cells, one can be recognised as the egg judged from its form and structure. The second cell has a distinct vacuole in the basal part below its nucleus and seems to be the only synergid formed. Out of the five nuclei, three are found together in close association and indeed in the final stages of separation from each other. From their arrangement and association they, undoubtedly, seem to have arisen from the same nucleus in an amitotic manner. Out of the remaining two nuclei, the nucleolus of one is constricted and it seems to be on its way to divide amitotically. The situation seems to have arisen in the following manner :—First of all, as usual, two polar groups of four nuclei each should have been formed. Soon after, the antipodals are organised and the polar nuclei move towards the centre, meet each other and finally take their position together near the antipodal end. At about the same time, the egg cell and one synergid are differentiated at the micropylar end, out of the three nuclei left over there. The undifferentiated nucleus undergoes an amitotic division and then one of the two daughter nuclei, in its turn, follows suit. Thus, it ultimately leads to the formation of extra nuclei observed in the case recorded here.

Abnormalities in the structure of the embryo-sac have been previously noted in other flowering plants. They may be grouped as below :

(i) *Embryo-sacs that show fewer than* 8 *nuclei.*—These arise either due to the suppression of one or more divisions or degeneration of a few of the 8-nuclei formed (usually in the chalazal pole of the embryo-sac). Instances of this kind are noted in good many plants and *Oenothera* (4-nucleate) type is supposed to be formed from a total suppression of development of the chalazal group.

(ii) *Embryo-sacs that show* 8 *nuclei but with abnormal organization of their constituents.*—The abnormal organization of the constituents may involve loss of polarity, reversed polarity, want of differentiation



Figs. 19–29. *Thymelæa arvensis*.—Fig. 19. Primary archesporium. The epidermis of the ovule shows a periclinally divided cell. Fig. 20. M.M. cell

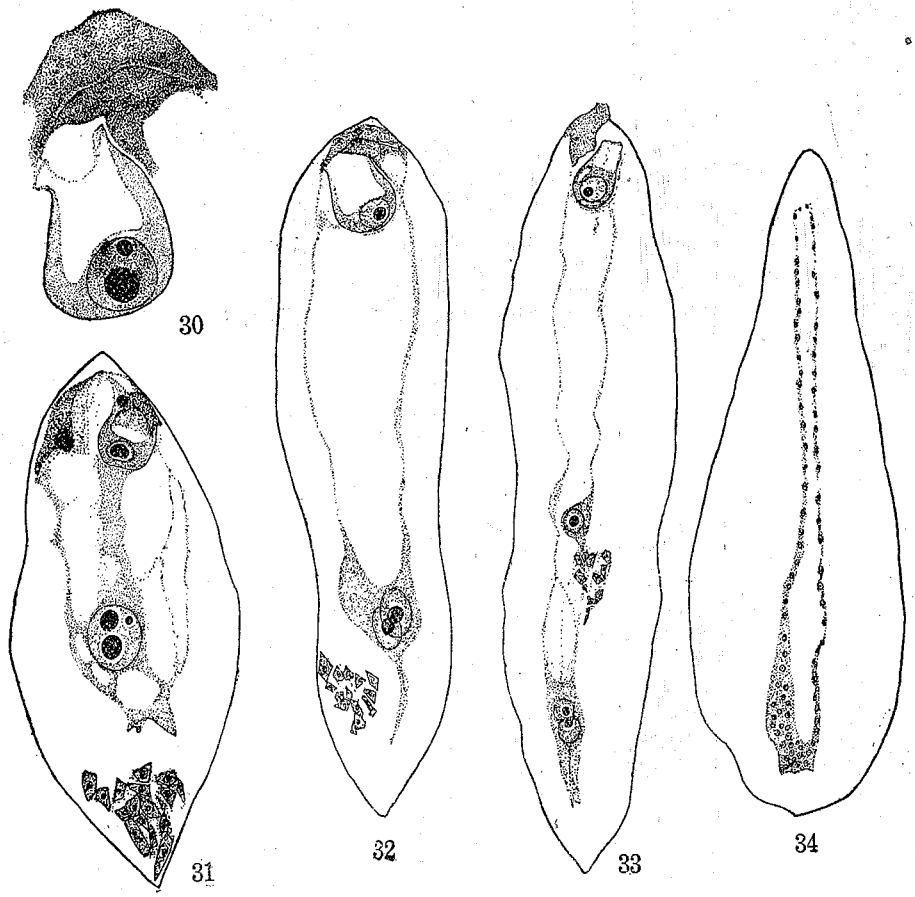
with two parietal cells above it. Fig. 21. L.S. of the nucellus of an ovule showing a linear tetrad of megaspores and nucellar cap formation. Fig. 22. 2-Nucleate embryo-sac. Figs. 23–27. Various stages in the embryo-sac development. Fig. 27. Shows a mature embryo-sac with many antipodals. Fig. 28. L.S. micropylar end of the embryo-sac showing the egg and a synergid. Fig. 29. An abnormal embryo-sac with extra nuclei. See text for further explanation. Figs. 19–21, 24–27 and 29, ×560; Figs. 22, 23 and 28, ×820.

in the constituents of the various groups, reduction in the number of members of one group and consequent increase in one or both the remaining groups, formation of extra egg cells at the expense of synergids, formation of extra number of synergids and eggs at the expense of other constituents, lack of differentiation in one or more constituents of either polar group, etc. Cases falling in this category are rare and always bear the character of abnormality.

(iii) *Embryo-sac with more than* 8 *nuclei.*—Extra nuclei arise due to secondary increase in the number of nuclei in either polar group. The extra nuclei so formed may add to the usual number constituting the egg-apparatus or polar nuclei or the antipodals. Secondary increase in the number of antipodals is found to occur as a normal feature in the embryo-sac of some plants, but secondary multiplication and increase in the micropylar group is very rare and always bears the character of abnormality.

The abnormal case of an embryo-sac in *Thymelæa arvensis* described above belongs to the last group. Schnarf (1929) and later Maheshwari (1941) enumerated important cases of abnormalities in the embryo-sac. Among these are included those that show extra nuclei. In 1880, Mellink recorded in *Luffa petiolata*, an embryo-sac divided into many cells and without any differentiation of either the antipodals or the egg-apparatus. In 1905, Shattuck reported occasional occurrence of super-numerary nuclei in some embryo-sacs of *Ulmus americana*, but recently, Fagerlind (1938), on a critical study of figures and statements published by various workers on *Ulmus* as well as his own preparations, suspects, that, in *Ulmus*, probably, a 16-nucleate embryo-sac is developed according to the *Drusa* form of the *Peperomia* type. In 1916, Dahlgren described abnormal cases of embryo-sacs in *Armeria alpina* and *A. plantaginea*. The embryo-sacs showed three synergids, one egg cell, three polar nuclei and five antipodals or four synergids, two egg cells, two polar nuclei and three antipodals. Next, Ekstrand (1918) recorded a few abnormal embryo-sacs in *Plantago major*. One of them showed seven cells in the egg apparatus, two polar nuclei and seven antipodals and another seven cells in the egg apparatus and three antipodals. In still another case, he found only three cells in the egg-apparatus and seven antipodals. Chiarugi (1925), working on *Tuberaria guttata*, found an abnormal embryo-sac with two synergids, each of them containing three big nuclei and one of them a satellite nucleus also in addition. The egg cell showed the normal form, but the secondary nucleus was divided into three nuclei. Modilewski (1925) recorded a few cases of embryo-sacs in *Allium odorum* showing three synergids, one egg cell, two polar nuclei and five or six antipodals. Maheshwari (1941) mentions a few more cases. According to him,

Gerassimova (1933), in *Crepis capillaris*, and Poddubnaja-Arnoldi and Dianowa (1934), in *Taraxacum kokosaghys*, noted occasional occurrence of 2–4 egg cells in addition to the other elements of the embryo-sac. Martinoli (1939) also occasionally found 9-nucleate and sometimes even 10-nucleate embryo-sacs in *Pyrethrum cinerariæfolium*. The author states that the extra nucleus in the 9-nucleate sac is formed due to a division of the egg and that it (extra nucleus) migrates to the centre of the embryo-sac. In the 10-nucleate sacs, he found three nuclei in the micropylar end, two in the centre of the sac (one of them being the additional cell derived from the division of the egg and the other being the nucleus formed by the fusion of the two normal polar



Figs. 30–34. *Thymelæa arvensis*.—Fig. 30. Egg fertilisation. Fig. 31. L.S. of embryo-sac in which the egg fertilisation is completed and the triple fusion is not yet completed. Fig. 32. Embryo-sac showing initial stages of endosperm formation. Fig. 33. Slightly advanced stage, the embryo-sac elongates very much, and the antipodals are left behind. Fig. 34. Nuclear endosperm. Fig. 30, ×1250 ; Fig. 31. ×560 ; Fig. 32, ×395 ; Fig. 33, ×265 ; Fig. 34, ×53.

nuclei) and five in the chalazal end. P. C. Joshi (1935) found in *Thylacospermum rupifragum* in the ovules of an abnormal ovary some abnormal embryo-sacs with 15, 16, 18 and 21 nuclei, in addition to 4- and 8-nucleate sacs. Usually in this species, an 8-nucleate embryo-sac is developed according to the normal type.

Obturator

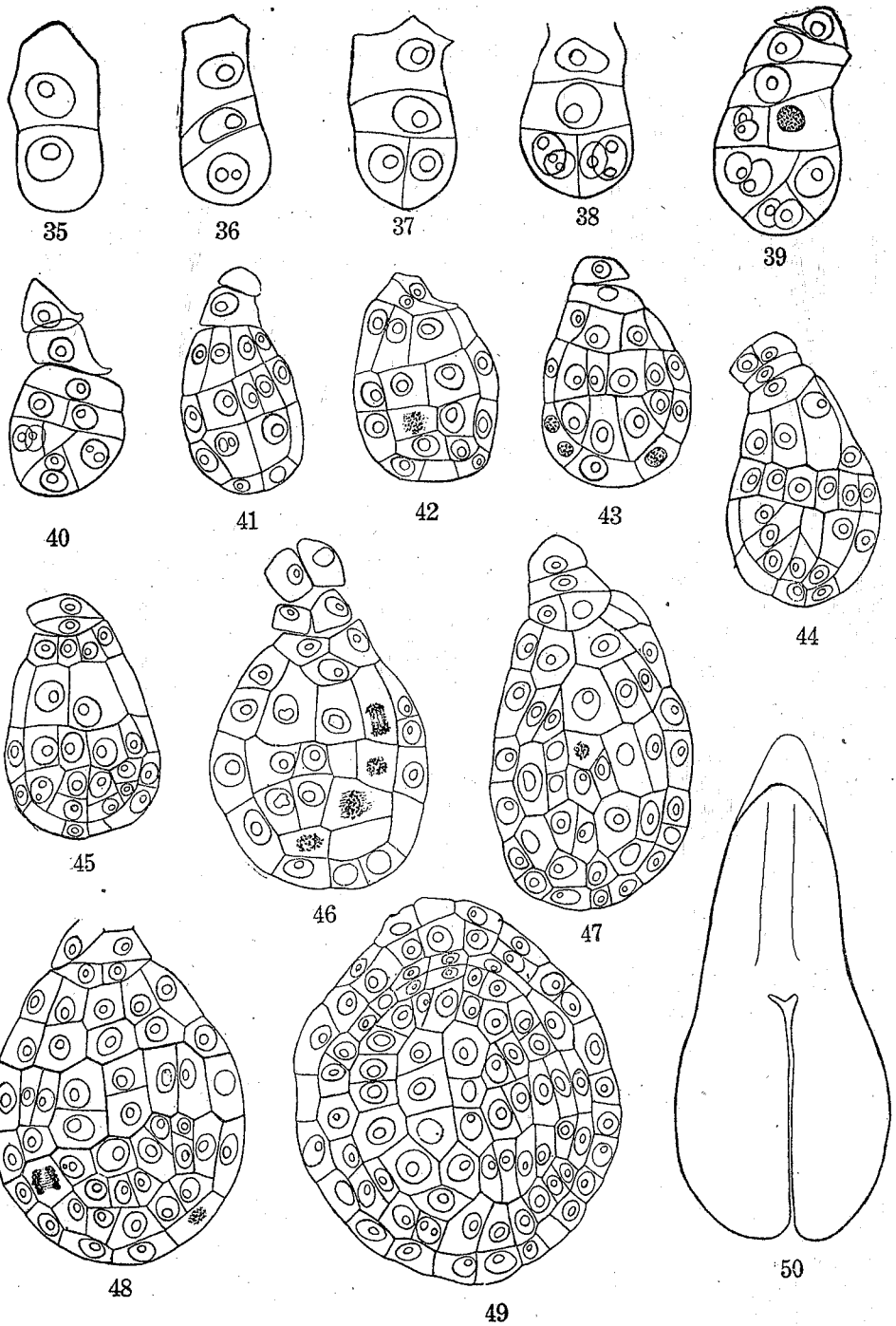
Early during the development of the ovule, the cells lining the basal part of the stylar tissue grow out into elongated cells and traverse the short space between the micropyle of the ovule and roof of the carpel (Figs. 15 and 16). All these elongated cells are rich in protoplasmic contents and form a more or less compact bundle converging towards the apex of the nucellus. The bundle descends down the funnel-shaped micropyle till it touches the nucellar apex. The presence of similarly developed obturators in other members of Thymelæaceæ has been known for a long time. They differ in their form, but not in origin. For instance, the obturator is more or less compact in *Thymelæa*, *Wikstræmia* and *Daphne*, while it is loosely arranged in *Peddia*, *Gnidia*, *Passerina*, etc. An obturator is developed also in the Elægnaceæ but it takes its origin from the funicle. An obturator is also present in Euphorbiaceæ, Rosaceæ, Umbelliferæ, etc., which are unrelated to each other. In all cases, however, it is undoubtedly concerned in directing the pollen tube to the micropyle.

Fertilisation

The mature pollen grains, when shed, are 3-nucleate. They germinate on the stigma and the pollen tubes run down through the style and obturator and reach the nucellus of the ovule. After entering the embryo-sac, the pollen tube seems to proceed towards one of the synergids and not directly to the egg cell. Double fertilisation and triple fusion occur. One sperm enters the egg cell and is seen to be within the egg nucleus for some time before actually fusing with it (Fig. 30). The fusion between the egg nucleus and one of the sperms precedes the triple fusion (Fig. 31). The two polar nuclei lie side by side for a long time and fuse just before triple fusion.

Endosperm

The endosperm is formed according to the nuclear type. After triple fusion, the endosperm primordium seems to divide near the antipodal end. The first division though not seen in my preparations, seems to be completed soon after the triple fusion and a few endosperm nuclei are always formed much before the division of the fertilised egg (Figs. 32 and 33). The nuclei migrate upwards and the cytoplasm, though scanty, accumulates in the micropylar and chalazal ends but remains thin on the sides. The chalazal accumulation is more prominent and the endosperm nuclei lie embedded in it (Fig. 34). The endosperm nuclei undergo further divisions and increase in number. The endosperm remains cœnocytic for a pretty long time, but ultimately becomes cellular (Fig. 18). The central vacuole, however is never filled up with cells.



Figs. 35–50. *Thymelæa arvensis*.—Various stages in the development of the embryo. Figs. 35–49, ×560; Fig. 50, ×53.

EMBRYO

The first division in the fertilized egg is transverse and takes place only after some endosperm nuclei have been formed. As a result of this division in the oospore, an apical cell and a basal cell are formed (Fig. 35). One more transverse division results in a 3-celled proembryo. No mitotic figure has been observed to enable me to say definitely whether it is the basal or the apical cell that undergoes the second division, but from thickness of the walls separating the three cells, it appears to have taken place in the apical cell (Fig. 36). Fuchs (1938) is also of the same opinion though no actual mitotic figure has been observed even in her preparations. Usually the proembryo remains 3-celled till the appearance of the first longitudinal wall dividing the apical cell (Fig. 37). Further increase in the length of the embryo takes place, always after the appearance of a longitudinal wall in the apical cell as a result of transverse division in either the basal or the middle cell of the proembryo. Due to lack of mitotic figures in the preparations, it is not possible to say definitely whether such further increase in the length of the embryo is due to transverse divisions in the basal or the middle cell of the proembryo.

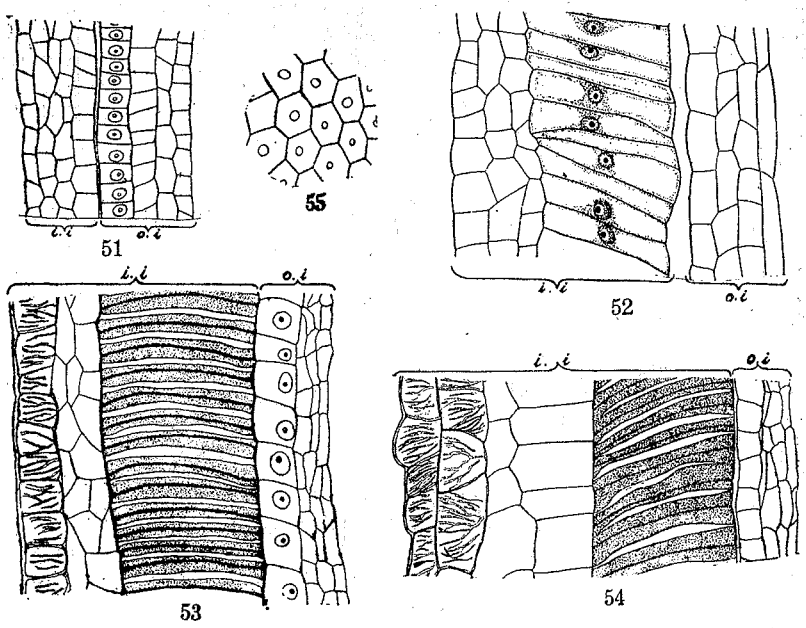
The embryo proper develops usually from four apical cells of the proembryo (Figs. 40–49). All these four cells, however, are not formed when the first longitudinal division takes place in the apical cell. The III and IV cells from the apex taking part in the formation of the embryo proper are formed at about the time when the sub-apical cell divides longitudinally or soon after it completes the division (Fig. 39). Such a belated differentiation of some cells taking part in the formation of the embryo-proper has been recorded by Kajale in *Boerhaavia diffusa* and *B. repanda* (Kajale, 1938). It is worthy of note that in the above referred species of Nyctaginaceæ also, four cells of the proembryo take part in the formation of the embryo proper (including hypophysis). The other families of Myrtales, in which detailed development of the embryo is known, differ from Thymelæaceæ in the fact that mainly the apical cell takes part in the formation of the embryo proper, the sub-apical cell only contributing to the formation of the hypophysis.

The further development of the embryo also resembles that described by Kajale (1938) in *Boerhaavia diffusa* and *B. repanda*. The four cells taking part form the different parts of the embryo as follows :—

The apical cell forms the cotyledons and the plumule. The second and third cells from the apex give rise to hypocotyl and the major part of the radicle (Figs. 46–49). The fourth cell forms the hypophysis which forms the apex of the radicle (Figs. 44, 46–49). A case however, showing that five cells may also take part in forming the embryo has been noted (Fig. 45). In such a case II, III and IV cell tiers from the apex take part in the development of the hypocotyle and radicle.

The four cells or the tiers to which they give rise do not develop simultaneously during the differentiation of the embryo. The apical-most is the first one to divide (Figs. 37, 38). Only when it forms the quadrants, the sub-apical one undergoes the first division in a longitudinal manner. It is soon followed by a longitudinal division in the third cell from the apex. By this time the fourth cell may not be even differentiated. The fourth cell is differentiated only when the dermatogen is formed in the third tier (Figs. 42, 44). Their further differentiation is given below :—

The apical cell undergoes the longitudinal division while the proembryo is only 3-celled (Fig. 37). Then one more longitudinal division in it in a plane at right angles to the first leads to the formation of the quadrants (Fig. 38). Till now, the other cells of the proembryo do not show either a transverse division or a longitudinal division. One of the quadrant cells in the apical tier undergoes an anticlinal division (Fig. 39) and the cells get arranged as if separated by oblique walls (Fig. 39). In some of the quadrants of the same tier periclinal divisions begin to take place resulting in the differentiation of the dermatogen (Fig. 40). At about this time the first longitudinal wall appears in the sub-apical cell (Fig. 40) and is soon followed by a longitudinal division in the third cell from the apex. Mostly before the first longitudinal division takes place in the third cell, the differentiation of the fourth cell taking part in the formation of the



Figs. 51–55. *Thymelæa arvensis.*—Figs. 52–54. Longitudinal sections showing various stages in the development of the seed-coat. Fig. 55. T.S. of woody palisade layer of the inner seed-coat. Figs. 51 and 52, ×373 ; Figs. 53 and 54, ×263 ; Fig. 55, ×373.

embryo proper takes place. The differentiation of dermatogen in the apical cell is soon followed by differentiation of dermatogen in the second and third tiers (Figs. 41, 43). Further multiplication of the cells inside the dermatogen takes place first of all in the apical tier in which first longitudinal or oblique walls are formed (Fig. 44). The cells so formed divide by transverse walls and result in two tiers of cells (Figs. 44–45). Similar multiplication of cells follows in the second and third tiers (Figs. 46 and 47). The cells of the second tier are shorter when compared to those composing first and third tiers (Figs. 44–46).

The differentiation of periblem and plerome follows that of dermatogen. The periblem and plerome are differentiated after a few periclinal divisions in the inner cells, while the dermatogen cells divide only in an anticlinal manner.

The cell lying next to the third cell (or tier) from the apex seems to divide transversely (Fig. 43). The daughter cell towards the apex forms the hypophysis and that towards the base adds to the length of the suspensor. The hypophysis divides anticlinally and periclinally and forms the apex of the root and the root-cap (Figs. 46–49).

The suspensor is very short and is usually made up of 2 or 3 cells (Figs. 44, 46). One, two or all of the three cells, in the later stages, may divide longitudinally and make the suspensor partly or wholly 2-seriate (Figs. 44 and 46). At about the time of the completion of the root-apex or slightly before that time, the cells of the suspensor become loose from each other (Fig. 46). Usually the suspensor cannot be seen in the advanced stages of the embryo. The embryo then grows in size. The cotyledons and stem apex are differentiated at the apex. The embryo is straight (Fig. 50).

Structure of the Seed-coat

During its development, the embryo destroys the whole of the endosperm and later on the nucellus, but one or two layers of nucellus persist. So there is present a vestige of perisperm in the seed. As already mentioned, due to their growth in the micropylar region, the integuments come together and the funnel-shaped micropyle becomes narrow.

To start with the integuments are mostly three cells in thickness. In the fertilised ovules, each of them becomes four cells in thickness (Fig. 51). The inner epidermis of the outer integument consists of prominent cells which have rich protoplasmic contents (Fig. 51). In later stages of development the cells of the outer epidermis of the inner integument become very much elongated (Fig. 52). In the seed, cells of this layer become very much thickened and their cavities are greatly reduced (Figs. 53, 54 and 55). The cell walls also become lignified and the cells form a woody palisade layer (Figs. 53 and 54). The cells composing one or two of the innermost layers of the inner seed-coat develop fibrous thickenings on their walls such as are seen in the cells of endothecium in the anther wall. The cells composing the outer

seed-coat become greatly stretched and form the thin outer seed-coat (Fig. 54).

Summary

The structure and development of the anther, pollen, ovule, embryo-sac, endosperm, embryo and seed-coat of *Thymelæa arvensis* Lamk. are described.

The anther development follows the normal course. The wall of the anther at first consists of the epidermis, endothecium, a single middle layer and the tapetum. The epidermis is greatly thinned out due to stretching in mature anthers and the single middle layer between the tapetum and the endothecium gets crushed. The primary sporogenous cells usually become the pollen mother cells. The pollen grains at the shedding stage are 3-nucleate. The sperms are elongated. Starch is present in the pollen grains. The exine shows reticulate sculpture and many germ pores. Two cases of pollen grains with more than three nuclei are described.

The solitary ovule is anatropus and two-integumented. The micropyle is formed by the inner integument. The epidermal cells of the nucellus undergo periclinal divisions and form a 2–3 cells thick cap. The archesporium in the ovule consists of a solitary sub-epidermal cell, which cuts off a primary parietal cell. The latter forms two-layers of parietal tissue which are crushed later by the developing embryo-sac. A linear tetrad of megaspores is formed. The chalazal-most megaspore gives rise to an 8-nucleate embryo-sac according to the normal type. The antipodals increase in number to about 25–30 and persist, although in a degenerate state, a long while after fertilisation. An exceptional embryo-sac with more than 8 nuclei is described.

There is a chalazal conducting strand of elongated cells in the nucellus. An obturator is developed from the base of the style. This descends down into the micropyle and helps to lead the pollen tube towards the embryo-sac.

Fertilisation is porogamous. Double fertilisation and triple fusion occur.

Endosperm is formed according to the nuclear type, but becomes cellular in the later stages. It is completely consumed by the embryo in the mature seed.

Embryo development has been followed in detail. A 3-celled proembryo is formed at first. Four cells take part in the formation of the embryo (including hypophysis), as in *Boerhaavia*. All these four cells, however, are not differentiated at the same time. Only after the apical cell of the embryo completes the first longitudinal division and forms the quadrants the third and fourth cells taking part in the formation of the embryo are differentiated. The apical cell forms the cotyledons and the plumule. The sub-apical cell and the one below it form the hypocotyl and large part of the radicle, while the fourth cell forms the apex of the root and the root-cap.

The seed-coats are two in number. The outer is 4 cells in thickness and membranous. The inner is usually 5 cells thick. Its outermost layer consists of thick-walled palisade cells. The middle cell layers are parenchymatous. The cells of the two innermost layers develop fibrous thickenings.

In conclusion, I wish to experss my sincere thanks to Prof. A. C. Joshi, D.Sc., F.N.I., for his helpful suggestions and criticism during the investigation. I am also indebted to him for the material used in this investigation.

LITERATURE CITED

Beauregard, M. (1877) .. "Structure et développement du fruit des *Daphne*," *Bull. Soc. bot. France*, **24**, 385–87 (Quoted in Fuchs, 1938).

Bhargava, H. R. (1936) .. "The Life-history of *Chenopodium album* Linn.," *Proc. Ind. Acad. Sci.*, **B 4**, 3, 179–200.

Brongiart, R. (1826) .. "Zeugung des Pflanzenembryos," *Browns-Verm Bot. Schr.*, **4**, 234–317 (Quoted in Fuchs, 1938).

Capus, G. (1878) .. "Anatomie du tissue conducteur," *Ann. Sci. nat. Bot.*, Ser. **6**, 7, 209–91 (Quoted in Fuchs, 1938).

Coulter, J. Mc., and Chamberlain, C. J. (1903) *Morphology of Angiosperms*, New York.

Dahlgren, K. V. O. (1915) .. "Uber die Uberwinterungsstadien der pollen–säcke, und der samenanlagen einiger Angiospermen," *Svensk. Bot. Tidskr.*, **9**, 1–12 (Quoted in Fuchs, 1938).

Dutt, N. L., and Subba Rao, K. S. (1933) "Observations on the cytology of the sugarcane," *Ind. Jour. Agr. Sci.*, **3**, 37–56.

Fuchs, A. (1938) .. "Beitrage zur embryologie der Thymelæaceæ," *Osterr. Bot. Zeitschr.*, **87**, 1, 1–41.

Guèrin, P. (1913) .. "Lé integument séminal et lés trachees nucellaires des Thymélaeacēes," *C. R. acad. Paris*, **156**, 398–400.

——— (1915) .. "Reliquiæ Treubianæ. I. Recherches sur la structure de l'ovule et de la graine des Thymélaéacées," *Ann. Jard. bot. Buitenzorg*, **29**, 3–33.

Hofmeister, W. (1849) .. *Die Enstehung des embryos der Phanerogamen*, Leipzig (Quoted in Fuchs, 1938).

Joshi, A. C. (1937) .. "A study of pollen in the Thymelæaceæ," *Proc. Ind. Sci. Congress*, **24**, Section 4 (Botany Abstracts).

—— and Venkateswarlu, J. (1935) "Embryological studies in the Lythraceæ. I. *Lawsonia inermis* Linn.," *Proc. Ind. Acad. Sci.*, **B 2**, 5, 481–93.

——— (1935 *b*) .. "Embryological studies in the Lythraceæ. II. *Lagerstræmia* Linn.," *ibid.*, **6**, 523–34.

——— (1936) .. "Embryological studies in the Lythraceæ, III," *ibid.*, **3**, 5, 377–400.

Joshi, P. C. (1935) .. "Some abnormal ovules and embryo-sacs of *Thylacosperm rupifragum* Shrenk," *Curr. Sci.*, **3**, **11**, 560–61.

——— (1936) .. "Contribution to the life-history of *Stelleria media* L.," *Proc. Ind. Acad. Sci.*, **B 3**, 8–22.

Kajale, L. B. (1938) .. "Embryo and seed-development in the Nyctaginaceæ. I. Studies in the genus *Boerhaavia*," *Jour. Ind. Bot. Soc.*, **4**, 243–54.

Kajale, L. B. (1940) .. "A contribution to the embryology of Amarantaceæ," *Proc. Nat. Inst. Sci.*, **6**, 4, 597–625.

Kausik, S. B. (1940) .. "Structure and development of the ovule and embryo-sac in *Lasiosiphon eriocephalus* Dcne," *ibid.*, **1**, 117–32.

Maheshwari, P. (1941) .. "Recent work on the types of embryo-sacs in angiosperms—A Critical Review," *Jour. Ind. Bot. Soc.*, **5 and 6**, 229–61.

Mauritzon, J. (1939) .. "Contributions to the embryology of the orders, Rosales and Myrtales," *Lunds univ. Arsskrift. N.F. And.*, **2**, 35, 120.

Osawa, I. (1913) .. "On the development of the pollen grain and embryo-sac of *Daphne* with special reference to the sterility in *Daphne odora*," *Jour. Coll. Agr. Tokyo*, **5**, 4, 237–64.

Prohaska, K. (1883) .. "Der Embryo sack und die Endosperm bildung in der gattung *Daphn.*," *Bot. Ztge.*, **41**, 865–68. (Quoted in Osawa, 1913, and Fuchs, 1938).

Schnarf, K. (1929) .. *Embryologie der Angiospermen*, Berlin.

——— (1931) .. *Vergleichende Embryologie der Angiospermen*, Berlin.

Stephens, E. L. (1909) .. "The Embryo-sac and embryo of *Geissoloma marginata*," *New Phyto.*, **8**, 345–48.

Strasburger, E. (1884) .. "Die Endospermbildung bei *Daphne*," *Ber. d. deutsch. bot. Ges.*, **2**, 112–14 (Quoted in Osawa, 1913, and Fuchs, 1938).

——— (1885) .. "Zu *Santalum* und *Daphne*," *ibid.*, **3**, 105–13 (Quoted in Osawa, 1913, and Fuchs, 1938).

——— (1909) .. "Zeitpunkt der Bestimmung des Geschlechts, Apogamie, Partheno-genesis und Reduktionsteilung," *Histol. Beitr.*, Heft **7**, Jena (Quoted in Fuchs, 1938, and Schnarf, 1931).

——— (1910) .. "Chromosomenzahl," *Flora*, **100**, 330–46 (Quoted in Schnarf, 1931).

Venkateswarlu, J. (1937*a*) .. "Structure and development of the embryo-sac of *Pemphis acidula* Forst.," *Jour. Ind. Bot. Soc.*, **16**, 5, 259–62.

——— (1937 *b*) .. "A contribution to the embryology of Sonneratiaceæ," *Proc. Ind. Acad. Sci.*, **B**, 206–23.

Vesque, J. (1879) .. "Nouvelles recherches sur le développement des phanerogames angiospermes," *Ann. Sci. Nat. bot.* Ser. **6**, 8, 261–390 (Quoted in Fuchs, 1938).

Winkler, H. (1904) .. "Die Parthenogenesis bei *Wikstræmia indica* (L.) C. A. Mey," *Ber. d. deustch. bot. Ges.*, **22**, 573–80 (Quoted in Schnarf, 1921).

——— (1906) .. "Botanische Untersuchungen aus Buitenzorg. II. Uber Parthenogenesis bei *Wikstroemia indica*," *Ann. Jard. Buitenzorg*, **2**, 5, 208–76 (Quoted in Fuchs, 1938, and Schnarf, 1931).

Yamaha, G. (1926) .. "Uber die zytokinese beider pollen tetradenbildung zugleich weitere Beiträge. Zur kenntnis über die zytokinese im, Pflanz enrieche," *Jap. Jour. Bot.*, **3**, 139–62 (Quoted in Schnarf, 1931, and Fuchs, 1938).