STRUCTURE AND DEVELOPMENT OF THE OVULE AND EMBRYO SAC OF *LASIOSIPHON ERIOCEPHALUS* DCNE.

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INTRODUCTION.

The earliest embryological investigation on the Thymelaeaceae is by Hofmeister in 1849 on *Daphne* and since then a number of contributions have appeared from time to time. Schnarf (1931) has reviewed these in his treatise on the angiosperms and has given the following summary of the embryology of the family.

The development of pollen follows the simultaneous type. In *Daphne* a transitory cell plate is formed during the heterotypic division. The mature pollen contains three nuclei. The ovule has a massive nucellus with two integuments. According to Guérin (1913, 1915) there is a remarkable development of the nucellar vascular bundle in a few members of the Thymelaeaceae, namely, *Dicranolepis, Craterosiphon* and *Synaptolepis*. The archesporium consists of a single cell which divides to give rise to a parietal cell and the megaspore mother cell. The development of the embryo sac follows the normal type. The antipodals are variable; they are small in *Wickstroemia indica, W. canascens, Gnidia carinata* and *Daphne alpina*, but show an increase in number in *Daphne odora* (three to six), *Blagayana pseudomezereum, Thymelaea passerina, Dirca palustris* (numerous) and *Daphnopsis Schwartzii* (many or three). In most species an obturator develops at the base of the stylar canal for the conduction of the pollen tubes towards the micropyle. The endosperm is free nuclear. Abnormalities occur in the family : the phenomenon of sterility in *Daphne odora* (Osawa, 1913) and somatic parthenogenesis and associated nucellar embryony in *Wickstroemia indica*.

Since the publication of Schnarf's work some further investigations have been made recently. Joshi (1937) has studied the development of the pollen in *Thymelaea arvensis, Wickstroemia indica* and *Daphne Mezereum*. Fuchs (1938) has made a detailed study of the embryology of some members of the family and has also discussed the affinities.

The present paper is based on a morphological and embryological study of *Lasiosiphon eriocephalus* Dcne. with particular reference to some important features in the development of the embryo sac and in the structure of the ovule.

Material and Methods.

The material for investigation was collected from the forests of the Western Ghats in the Mysore State during the month of December, 1938. Bouin's fluid was largely used for fixation. In addition, material preserved in formalin-acetic acid-alcohol was also made use of. The regular methods of dehydration and infiltration were followed. In some cases the material was run through mixtures of alcohol and chloroform and infiltrated in pure chloroform. By this method many of the difficulties in sectioning the material, especially in old stages when the floral parts become hard, were overcome to a certain extent. All the sections were stained in Heidenhain's iron-alum haematoxylin which was quite satisfactory.

The Flower.

The flowers are grouped together in dense head-like inflorescences and develop according to a racemose plan. Each flower has a single whorl of perianth which forms a long tube (fig. 3), at the top of which are seen five small imbricating segments of the perianth. The outer surface of the perianth is densely clothed over by numerous silky hairs. In very advanced stages of the flower the upper half of the perianth becomes detached by the formation of an abscission layer and the lower half remains as a loose membranous covering for the developing fruit.

There are ten stamens in two alternating whorls of five each and these are adnate to the perianth. The vascular connections to the outer (upper) whorl of stamens are separated from the midrib bundles of the perianth, while those to the inner (lower) whorl are separated from the combined lateral strands supplying the adjacent margins of the perianth segments. Outside the outer whorl of stamens and alternating with these and the perianth segments there are five fleshy parenchymatous scale-like structures which are roughly bifid (fig. 4). These do not have any vascular connections and may, therefore, be regarded merely as lobes formed by the perianth segments.

The ovary is raised on a short stalk and contains a single ovule. The style is long and narrow and terminates in a head-like stigma (fig. 5). The peripheral epidermal cells of the stigma become conspicuous very early with rich contents and gradually form a number of papillae (figs. 6, 7). These papillae next become elongated considerably to form short hairs (fig. 8) amidst which the pollen grains are deposited. After the receptive function is over the stigmatic hairs are filled with tannin and later may either persist as such or may drop off.

The external surface of the ovary is covered over by hairs similar to those found on the perianth. These hairs gradually fall off later as the fruit begins to be formed.

At the base of the ovary a conspicuous ring-like disc-scale develops. This is provided with vascular connections from the receptacular stele (fig. 3).

This disc-scale is regarded as a much reduced inner whorl of floral leaves, the corolla, as suggested by Joshi (1936) in another member of the Thymelaeaceae, *Stellera chamæjasme*.

Development of the Microsporangium.

The primordia for the two sets of stamens are formed very early in the ontogeny of the flower. They are at first made up of uniform cells without any differentiation. As further growth takes place, a single layer of cells becomes clearly marked out within the epidermis (fig. 10). This layer constitutes the archesporium of the anther which next divides periclinally to form an outer primary parietal layer and an inner sporogenous layer. The former divides again periclinally immediately to form two layers (fig. 11), of which the outer without further divisions functions as the endothecium in the mature anther, while the inner divides for the last time periclinally to give rise to the single middle layer and the tapetum (fig. 12). The tapetum lies in immediate contact with the sporogenous layer, the cells of which have divided in the meanwhile to form two layers of microspore mother cells.

During the development and increase in the size of the tapetal cells and the spore mother cells, both the endothecium and the single middle layer are greatly crushed tangentially (fig. 13). With the increase in the size of the tapetal cells, which marks the commencement of their activity, the nucleus of each cell divides once mitotically so that the tapetal cells finally become binucleate. At this time the cells are more or less bulging with their inner walls rounded off (fig. 13).

Just as a tapetal layer is formed towards the outside of the sporogenous cells, it is also formed towards the inside by the cells of the connective tissue of the anther. These inner tapetal cells are exactly similar to the outer ones both in shape, as well as in contents, being also similarly binucleate (fig. 13). In the older stages of the anther, the tapetal cells break down and their disorganised remnants may be seen as darkly staining scattered bits within the wall of the anther.

Development of the Megasporangium and the Female Gametophyte.

As already stated, the ovary contains a single ovule which is attached laterally on the placenta with the micropyle pointing upwards (fig. 9). The ovule first develops as a mass of undifferentiated tissue and gradually assumes its typical structure by forming two annular rings of tissue one below the other (figs. 3, 15). These are the primordia of the two integuments. At this stage a large space is seen developing in the cavity of the ovary below the ovule (fig. 3). This space persists characteristically in subsequent stages and although an attempt was made to verify if this represented any provision for the development of a second ovule, no evidence for this was forthcoming. In later stages, when the seed begins to develop after fertilisation, the space is

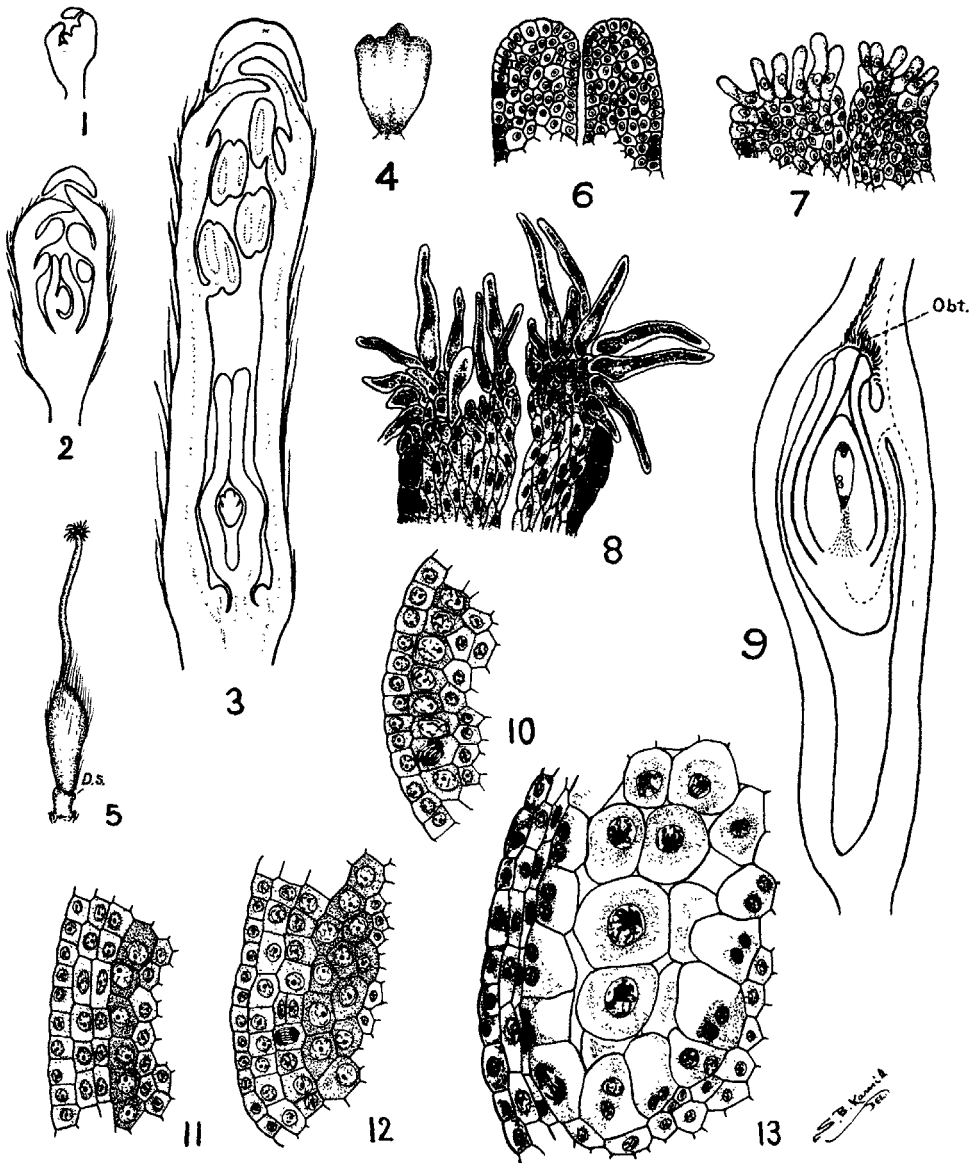
partly filled up by the downward growth of the base of the seed, while a small portion of it still remains and becomes filled with air.

During the development of the ovule, the micropyle comes in contact with a special tissue formed at the base of the stylar canal (fig. 9). This tissue consists of regularly arranged elongated cells containing abundant material and projecting into the ovarian cavity (fig. 14). This is the obturator which functions as a device for the conduction and growth of the pollen tubes as they descend from the stylar canal towards the micropyle. The obturator is characteristic of most of the Thymelaeaceae and it is also met with in certain other families, as Euphorbiaceae, Rosaceae, Elaeagnaceae, Umbellifereae, etc. The morphology of the obturator, however, is different in the several cases, for it arises either from the placenta or the funiculus, while in the Thymelaeaceae it is a specialised part of the transmitting tissue which fills the stylar canal.

After the inception of the integument primordia, both the integuments, which in most subsequent stages of development are free from each other, grow at first uniformly all round the nucellus (fig. 16). But after a time the inner integument grows more rapidly than the outer and alone forms the micropyle (fig. 9). The upper part of the inner integument at the region of the micropyle becomes conspicuous as a conical projection surmounted by a knob formed by the flaring rim of the integument (fig. 9). The rim of the outer integument stops short behind the knob and invests the part below the knob as a tight-fitting collar. Later, however, as the seed develops the outer free portion of the rim of the outer integument, namely that lying on the opposite side of the placenta, grows beyond the knob and, closely arching over, forms a hood-like covering for the micropyle (fig. 20).

Both the integuments are at first made up of three layers of cells except at the base where there may be four layers (fig. 15). Gradually the number of cell layers in the inner integument increases to four, and the cells, filled with dense cytoplasm, show a very regular arrangement (fig. 21). In the meanwhile the outer integument forms five layers of regularly arranged cells, which are not only larger than those of the inner integument, but also contain less cytoplasm and include vacuoles (fig. 21). As further growth of the ovule proceeds, the inner integument also becomes five layered, but all the cells, except those forming the inner epidermis, now contain only a little cytoplasm and extremely reduced nuclei (fig. 21). This evidently means that the materials once stored in the cells of the inner integument have passed into the nucellus for its growth. At this time the cells of the outer epidermis of the inner integument become radially elongated; and still later, when the seed develops, appear as a palisade layer made up of columnar cells (fig. 23). This feature is especially marked at the base of the integument.

Simultaneous with the changes in the inner integument as described above, the outer integument also shows further transformations. The number of cell layers remains as before, namely five, but the cells lose their regular



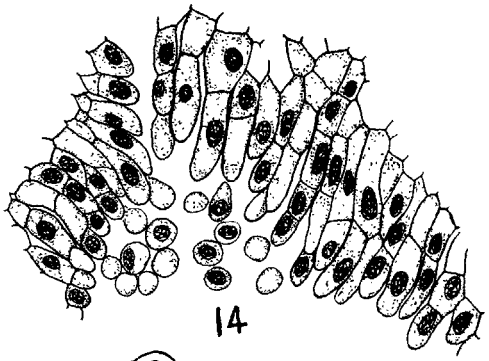
FIGS. 1–13. Figs 1–3. Stages in the development of the flower. ×40. Fig. 4. Scale-like lobe of the perianth. Fig. 5. Entire ovary showing disc-scale, *D.S.* ×6. Figs. 6-7. The stigmatic hairs forming as papillae. ×200. Fig. 8. Same, later stage showing tannin in the hairs. ×120. Fig. 9. Longitudinal section of ovary showing the position of the ovule and the obturator. ×40. Figs. 10–13. Stages in the development of the anther wall. ×450.

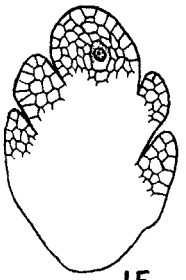
arrangement. Further, the cells of the inner layer become tannin-filled and take a brownish stain with haematoxylin (fig. 23). In the developing seed, the outer integument becomes very much compressed and forms a thin but fibrous covering for the seed.

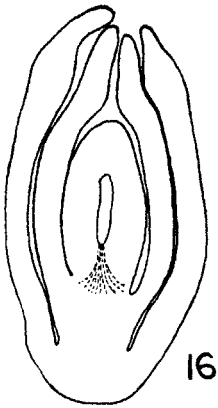
After the formation of the primordia of the integuments the inner mass of tissue, the nucellus, begins to grow rapidly. The apex of the nucellus assumes a more or less conical shape and the epidermal cells of this region begin to undergo a series of periclinal divisions to form the so-called epidermal cap (fig. 17). Below this cap a large amount of tissue with rich contents is recognisable. This tissue is designated by Fuchs (1938) as the 'Deckzellenkomplex' and is derived by the divisions of the primary parietal cell formed by the archesporium. This tissue, together with the overlying epidermal cap, gives a somewhat glandular appearance to the apex of the nucellus (*cf.* Proteaceae, Brough, 1933; Kausik, 1938*a*, 1938*b*, 1939), and is perhaps of advantage in the passage of the pollen tubes towards the embryo sac which is situated far below. The cells of the nucellus below the apex are also fairly well filled with materials although not to the same extent. But at the base of the nucellus and immediately below the antipodal end of the embryo sac a region of extremely rich cells develops conspicuously which are elongated and regularly arranged to form a strand of tissue (figs. 18, 19). This strand functions as a conducting strand for supplying materials to the embryo sac for its growth, not only prior to fertilisation, but also afterwards when the embryo sac elongates remarkably. The conducting strand spreads out at the base of the nucellus like a pedestal surmounted by the embryo sac.

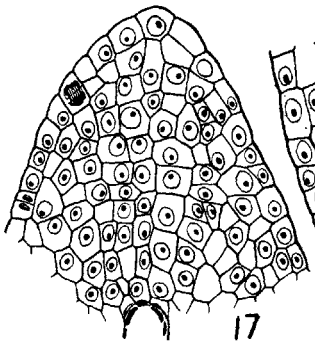
Fuchs (1938) describes the presence of a conducting strand in her study of the other members of the Thymelaeaceae. It is also met with in the Lythraceae (Joshi and Venkateswaralu, 1936) and in the Geissolomataceae (Stephens, 1909*a*). In the latter instance it appears to offer a certain amount of obstruction to the antipodal end of the embryo sac during its post-fertilisation elongation and is left over as a bundle of tissue projecting into the embryo sac when the latter grows down and all round it. Kershaw (1909) states that a conducting strand is present in the ovule of *Myrica Gale* and remarks that it may either represent the remains of an ancient nucellar vascular system or that it may be an entirely new structure developing at the base of the nucellus. In the Thymelaeaceae Guérin (1913, 1915) has found the nucellar tracheal tissue in some members, but it is absent in the other members studied by Fuchs (1938) and also in *Lasiosiphon*. It may, therefore, be suggested that the conducting strand, which is characteristic of the Thymelaeaceae and is also met with in other families which may not be related to one another on other grounds, may be regarded as a special device formed at the base of the nucellus and in response to the nutritive demands made by the embryo sac.

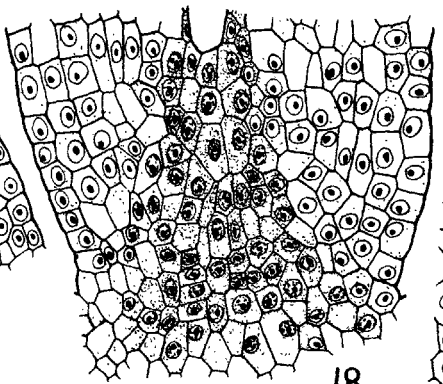
The female gametophyte.—It is hard to detect the presence of the archesporial cell in the nucellus of the young ovule as all the cells are similar. But in slightly older ovules, when the integuments have just been formed, there is

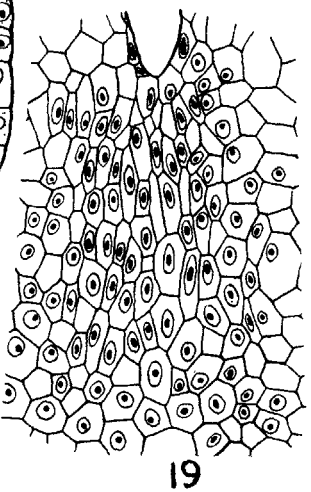


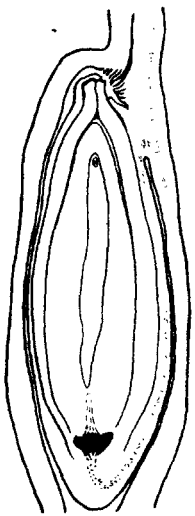


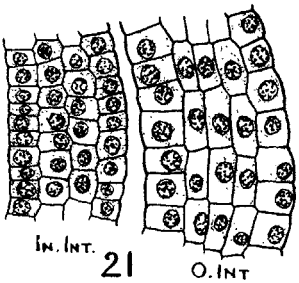


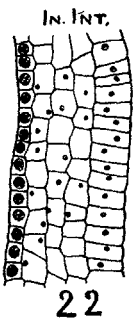


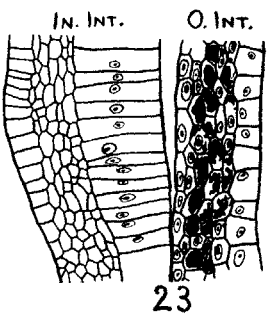








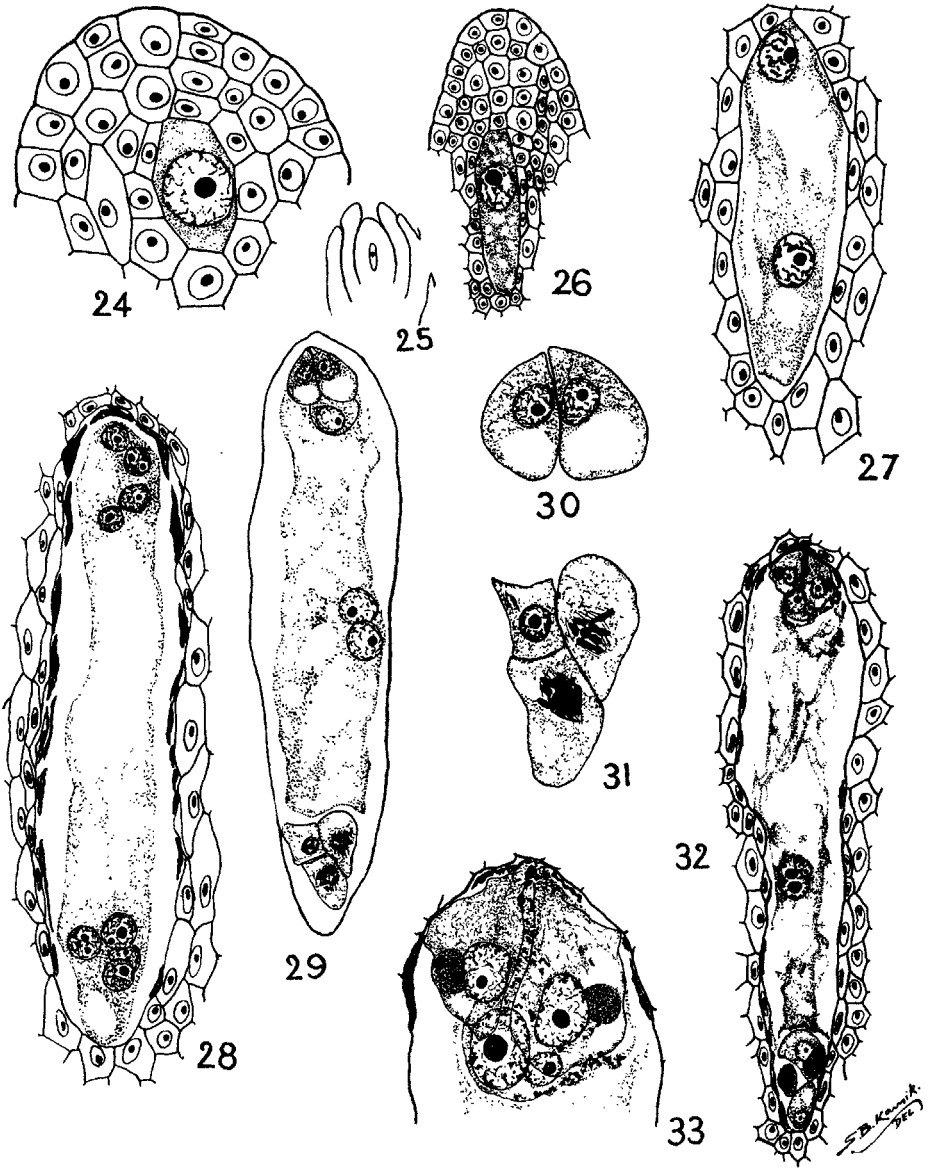




FIGS. 14–23. Fig. 14. Base of the stylar canal showing the obturator. ×400. Fig. 15. Young ovule showing the megaspore mother cell and the origin of the integuments. ×200. Fig. 16. Same, later stage showing the conducting strand at the base of the nucellus. ×200. Fig. 17. Apex of the nucellus showing formation of epidermal cap and the massive parietal tissue. ×400. Figs. 18, 19. Base of nucellus showing conducting strand. ×400. Fig. 20. Longitudinal section of seed with hypostase marked black. ×20. Fig. 21. Part of the integuments to show details. ×400. Fig. 22. Part of inner integument later. ×200. Fig. 23. Part of both integuments from the seed. ×200.

already seen a single large cell which by its position and characteristics must be regarded as the megaspore mother cell (figs. 15, 24). Overlying this there are two cells which are evidently formed from the parietal cell which has divided once periclinally soon after its formation from the archesporial cell (fig. 24). At this stage some of the epidermal cells at the apex of the nucellus have divided to form the epidermal cap so that the megaspore mother cell lies four cells below the outer surface of the nucellus. The mother cell next elongates rapidly (fig. 26) before undergoing the two divisions in the formation of the linear tetrad. The actual stages leading to the development of the tetrad and the survival of one megaspore to form the embryo sac were not available. A considerable time seems to elapse before any activity sets in in the mother cell during which it undergoes a period of rest. The next stage available in development was one shown in fig. 27 where the two nuclei of the young embryo sac are already formed and these have taken up their respective positions at the two ends. At this stage some vacuoles are seen in the embryo sac and attention may be specially drawn to one such vacuole below the primary chalazal nucleus. This vacuole persists in later stages and is present even after all the eight nuclei are formed (fig. 28). It is interesting to note here that a chalazal vacuole has been figured by Mauritzon (1934) at the two-nucleate stage of the embryo sac in *Cuphea lanceolata* and *C. platycentra* and by Joshi and Venkateswaralu (1936) in some of the other members of the Lythraceae. The latter authors state that 'the binucleate embryo-sac, as in *Lagerstroemia*, is often characterised by the persistence of the chalazal vacuole for an unusually long period and sometimes this condition is seen even during the development of the 4-nucleate embryo-sac or after its formation'. They further remark that this feature and the early degeneration of the antipodals in the Lythraceae, besides a few other minor ones, 'have been regarded by Tischler (1917) and Mauritzon (1934) to indicate that the embryo-sac of the Lythraceae forms phylogenetically an intermediate stage between the 4-nucleate embryo-sac of the Onagraceae and the normal 8-nucleate embryo-sac'. On the same ground it is reasonable to postulate here on the probable relationship of the Thymelaeaceae with the Lythraceae, between which families there also appear to be other almost parallel structural features in the development of the ovule. In fact there is some justification for this, for according to Hutchinson (1926) the Thymelaeales are to be regarded as the 'increasingly woody apetalous relations of the Lythrales'.

After the establishment of the eight nuclei the organisation of the embryo sac begins with the formation of the egg-apparatus, the antipodal cells and the two polar nuclei (fig. 29). The cytoplasm of the embryo sac is highly vacuolate and becomes more so a little later. The synergids are at first roughly pear-shaped with a large basal vacuole and a dense mass of cytoplasm containing the nucleus at the apex (figs. 29, 30). As the embryo sac reaches the stage of fertilisation, the synergids show each a slight beak-like projection appearing as a hump on the free outer side (figs. 32, 33). The nucleus assumes a more



FIGS. 24–33. Fig. 24. Part of nucellus with megaspore mother cell with formation of epidermal cap and two parietal cells. ×900. Fig. 25. Ovule with megaspore mother cell later. ×80. Fig. 26. Part of same enlarged. ×450. Figs. 27, 28. Two and eight nucleate embryo sacs respectively with chalazal vacuole. ×900. Fig. 29. Fully formed embryo sac. ×400. Figs. 30, 31. The synergids and the antipodals from the same enlarged and showing division of nucleus in two antipodals. ×900. Fig. 32. Embryo sac at the time of fertilisation and triple fusion. ×450. Fig. 33. Micropylar end of same enlarged to show a male nucleus in contact with the egg nucleus and the special spherical body in each synergid; both synergids are intact and the pollen tube has entered behind and between these. ×1350.

or less central position, and in contact with this there is sometimes seen a dense spherical body stained brownish with haematoxylin in each synergid (fig. 33). It is rather hard to discuss the nature of this extra body, but the suggestion that it may probably represent some special substance stored up in the synergid to facilitate the entry of the pollen tube by its chemotactic influence does not seem to be wholly unwarranted. This suggestion is further strengthened by the fact that in such cases both the synergids are often left intact when the pollen tube enters the embryo sac and discharges its contents (fig. 33). The further fate of this body could not be studied and it is sufficient to mention that it disappears from view in later stages when the synergids begin to degenerate some time after fertilisation.

It may be mentioned here that the body mentioned in the synergids looks very much like the kinoplasmic mass seen in the egg cell of some gymnosperms. Land (1904) describes in the case of *Ephedra trifurca* that 'a conspicuous kinoplasmic mass lies at a little distance below the nucleus' of the central cell of the archegonium. He further remarks: 'In the earliest stages it is coarsely granular, and later becomes dense, and is larger and sharper in outline than the similar body which is so conspicuous in some of the pines and in *Thuja occidentalis*'.

The antipodals are three in number, but in some cases the nucleus in each cell divides once mitotically (figs. 29, 31), followed by the separation into daughter cells. Either all the antipodals take part in this division, or only one or two may divide, so that finally the number of antipodals in a mature embryo sac varies from three to six. After fertilisation all these cells begin to degenerate and in later stages are recognisable only as heavily stained specks at the lower end of the embryo sac.

The two polar nuclei meet each other at first in the centre of the embryo sac (fig. 29), but may subsequently be found anywhere in the lower end or even very near the antipodals. Their union is considerably delayed and seems to take place regularly just prior to triple fusion. The two polar nuclei in a half fused state, showing distinctly two independent chromatin masses and two nucleoli, were seen with the second male nucleus closely perched on them as shown in fig. 32. Fuchs (1938) remarks that the two polar nuclei wander towards the chalazal end and states that she could not observe their fusion in her materials.

During all the stages leading to the final organisation of the embryo sac the neighbouring cells of the nucellus are crushed to a large extent and appear as several scattered bits all round the embryo sac. Thus these cells not only afford extra space for the enlarging embryo sac but also supply it with materials that are stored up in the earlier stages.

Fertilisation.

Stages showing the actual entry of the pollen tube into the embryo sac were not seen in any of the preparations. But slightly advanced stages with

the discharged pollen tube were met with. In such cases one of the two synergids was intact, while the other had been destroyed by the pollen tube. The surviving synergid was seen persisting even in later stages when the embryo sac had formed a few free endosperm nuclei (fig. 35). Sometimes both the synergids were found to be quite intact even though the pollen tube had entered and the two male nuclei were in association with the egg nucleus and the polar nuclei (fig. 32). It was in such a case that the spherical body already referred to was noticed in the synergids and as suggested it is probable that it has a definite rôle to perform in the actual entry of the pollen tube passing between and behind the synergids as shown in fig. 33. In this figure, the disorganised remnants of the tip of the tube are clearly seen as a number of dark specks scattered in the vicinity of the egg-apparatus and marking the track of the pollen tube. Joshi and Kajale (1937) have shown that both the synergids are frequently intact during fertilisation in *Alternanthera sessilis* and in earlier literature such a condition has also been noted in *Salix* (Chamberlain, 1897) and *Silphium* (Merrell, 1900).

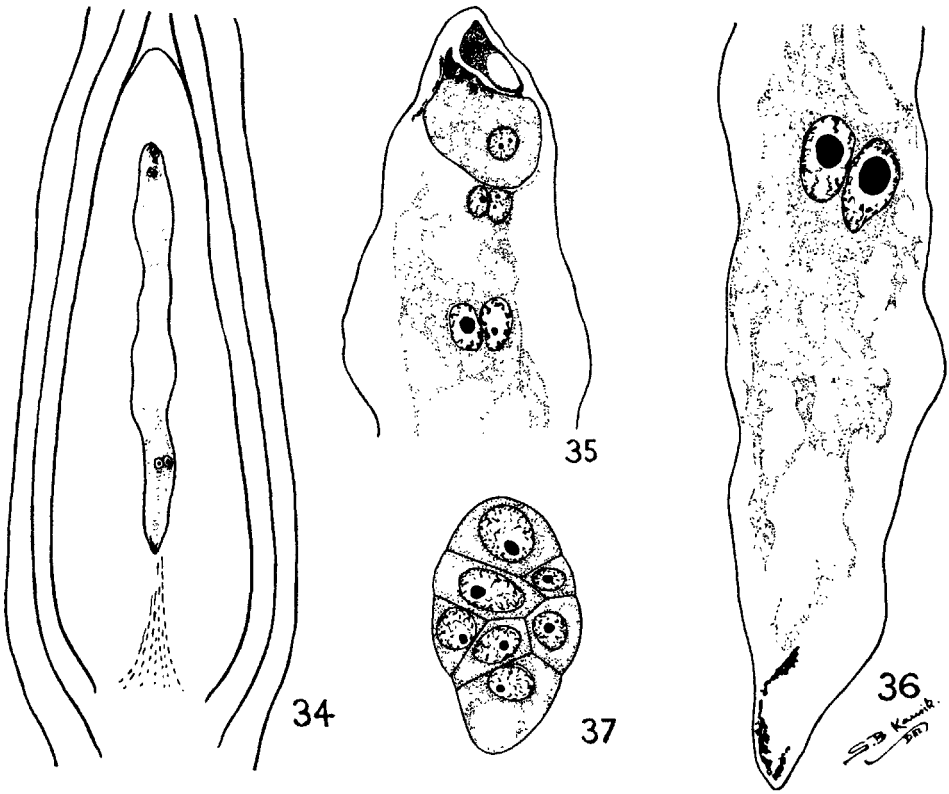
Fertilisation and triple fusion occur regularly in *Lasiosiphon eriocephalus*. The first male nucleus fuses with the egg nucleus, while the second travels down the embryo sac rapidly and fuses with the two polar nuclei which are uniting with each other just now. The actual fusion of the male nuclei seems to be simultaneous in both fertilisation and triple fusion.

Development of the Embryo and Endosperm.

The first division in the fertilised egg takes place only after a few free endosperm nuclei are formed. This division takes place by a transverse wall to form a proximal or basal cell and a distal cell. The second division, presumably also by a transverse wall, is formed in the distal cell so that the pro-embryo becomes three-celled as noted by Fuchs (1938). The further divisions are irregular and take place by inclining and interesting walls to form an oval embryo in which the distal and the proximal ends are clearly seen. There is a basal cell with large vacuoles (fig. 37). As further growth in the embryo proceeds the histogens are formed after which the embryo grows rapidly, encroaches on the endosperm and occupies the entire cavity of the seed in the mature condition.

The endosperm first arises in a free nuclear state and very early in development shows a paired arrangement of these nuclei at the micropylar, central, and chalazal portions of the embryo sac (figs. 34, 36). The pair of nuclei at the chalazal end is particularly conspicuous by their large size, and being embedded in a dense mass of cytoplasm (fig. 36) appears to take part to a considerable degree in the absorptive activity of the lower end of the embryo sac. Fuchs (1938) states that in later stages, when the endosperm becomes cellular, the lower end of the embryo sac contains large cells which have either many nuclei each or a single giant nucleus. The two large nuclei noted in the

present case probably give rise by further divisions to the giant nuclei described by her.



FIGS. 34–37. Fig. 34. Part of longitudinal section of seed to show a few free endosperm nuclei. ×40. Figs. 35, 36. Micropylar and chalazal ends to show the fertilised egg, one of the surviving synergids, and the paired arrangement of endosperm nuclei; the chalazal pair of nuclei is prominently seen in fig. 36. ×900. Fig. 37. A small many-celled embryo with rounded distal and wedge-shaped proximal ends. ×900.

After a large number of free nuclei are formed in the embryo sac, the endosperm tissue is built up which includes large and irregular cells. This tissue is rapidly encroached upon later by the growing embryo and is completely destroyed by it when the seed reaches the mature condition.

THE SEED.

The seed has a hard seed-coat formed by the two integuments. The outer integument forms a fibrous covering which may be easily peeled off from the seed, while the inner remains as a very rigid and firm investment for the

embryo. The outer epidermis of this integument forms a woody palisade layer (fig. 23) as mentioned by Fuchs (1938). The base of the seed shows a thick pad of tannin-filled tissue, the *hypostase*, which takes up a very heavy stain (fig. 20). Within the seed-coat, a thin layer of nucellus persists all around as a lining for the embryo, while the apex of the nucellus projects conically into the narrow micropyle.

Conclusions.

The present account of the embryology of *Lasiosiphon eriocephalus* Dcne. conforms in all respects to the earlier investigations in the family and includes a detailed study of some important features in the development of the ovule and the embryo sac. The noteworthy points in the life-history are the presence of the obturator, the formation of the epidermal cap and the cell complex arising from the parietal tissue in the ovule, and the presence of a conducting strand at the base of the nucellus. In the development of the embryo sac the presence of the chalazal vacuole has been pointed out and it has been suggested that in this feature, as well as in a few others, evidence for kinship between this family and the Lythraceae may be forthcoming. The presence of a special body in the synergids, which appears to be recorded here for the first time, can only be interpreted as a special feature of the synergids to help in the entry of the pollen tube into the embryo sac and its association in cases where both the synergids are left intact at the time of fertilisation lends additional support to this surmise.

The development of an oval embryo without a suspensor seems to resemble the condition in *Geissoloma marginata* (Stephens, 1909*a*) belonging to the Geissolomataceae and also found in the Penaeaceae (Stephens, 1909*b*). In the family Proteaceae also, which is regarded by some to be related to the Thymelaeaceae, it is curious to find a spherical (according to Ballantine, 1909 and Brough, 1933), but more correctly an oval, embryo (Kausik, 1938*a*, 1939). This fact, along with the presence of the glandular apex of the nucellus formed by the parietal tissue in both the Thymelaeaceae and the Proteaceae, appears to offer some ground for considering the relationship between the two families as really valid. From the point of floral anatomy also additional light seems to be thrown on this question, for in both the families the perianth represents the whorl of calyx (Joshi on *Stellera*, 1936; Kausik on *Macadamia*, 1938*b*, 1940), while the corolla is very much reduced and is represented by the disc at the base of the ovary. Thus the ancestral condition in both has to be regarded as a dichlamydeous one and the modern members have become simple through reduction. The arrangement of Bentham and Hooker (1862–1883) to include both these families under the Monochlamydeae must, therefore, be considered as unjustified.

Fuchs (1938) has discussed the systematic position of the family from an embryological standpoint and states that it is surprising that there are such

great differences between the closely related families Penaeaceae, Geissolomataceae and the Thymelaeaceae. It may be remarked here that these embryological differences may only mean individual specialisations in these several families and may not be fundamental in settling the affinities of these families. In the case of the Penaeaceae (Stephens, 1909*b*) it is true that there is the development of a 16-nucleate embryo sac by the participation of all the four megaspores, but Stephens suggests that it is a derived condition from, perhaps, the Geissolomataceae (1909*a*) which shows a normal development. Fuchs (1938) further discusses the systematic position of the Thymelaeaceae with the other families of the Myrtiflorae according to Engler and Gilg (1924) and states that in the possession of an obturator the family stands sharply distinct from all the others and points out that only the Elaeagnaceae, where a sort of an obturator is formed, come very close to the Thymelaeaceae.

Joshi (1938) has considered the affinities of the Thymelaeaceae from floral anatomy and points out from a study of *Stellera chamaejasme* that the arrangement of Hutchinson (1926) to include the family Nyctaginaceae also in the order Thymelaeales cannot be justified. He further states that the Thymelaeaceae must have come from dichlamydeous ancestors as the disc-scale represents a much reduced inner whorl of floral leaves, the corolla. The present writer is in complete agreement with this view. Joshi also proposes that the two subfamilies, the Aquilarieae and the Thymeleae, may be brought closer together on account of anatomical evidence for a bicarpellary gynoecium in *Stellera* belonging to the Thymeleae, which is generally regarded as possessing a monocarpellary gynoecium. Fuchs (1938) states that while the gynoecium in the family is usually made up of a single carpel, the Aquilarioideae and the Phalerioideae possess two carpels, while *Octolepis* has four. It, therefore, seems quite tenable that a reduction in the number of carpels has taken place in the family and that ancestrally there were more carpels. As an extensive study of the floral anatomy of *Lasiosiphon* does not fall within the scope of the present investigation, a more detailed approach to this question cannot now be made.

Summary.

1. The structure of the flower and the development of the anther and the ovule are described in detail.

2. The wall of the anther has four layers: the epidermis, endothecium, a single middle layer, and the tapetum. All these layers except the epidermis are formed from the primary parietal layer. The tapetal cells become binucleate and similar cells are also formed by the cells of the connective tissue of the anther towards the sporogenous layer.

3. In the developing ovule the apex of the nucellus becomes conspicuous with the formation of the epidermal cap and the cell complex arising from the primary parietal cell. There is a conducting strand at the base of the nucellus for supplying nutrition to the growing embryo sac.

4. The megaspore mother cell is formed by the archesporial cell after a parietal cell is cut off. The further behaviour of the mother cell is normal and the embryo sac develops according to the usual type.

5. A chalazal vacuole is seen in the developing embryo sac even after the 1-nucleate stage and its significance is considered in the light of previous literature.

6. The synergids sometimes show a spherical body which is regarded as a special substance to aid in the entry of the pollen tube into the embryo sac. Both the synergids are then found intact when the pollen tube enters, while at other times when the body is not seen one of the two synergids degenerates as usual.

7. The first division of the fertilised egg takes place only after a few free endosperm nuclei are formed. The embryo becomes oval in form during development and lacks a suspensor.

8. The endosperm is at first free nuclear and later forms a loose tissue made up of large and irregular cells. During the free nuclear condition two large nuclei are found at the chalazal end and they are probably concerned actively in the haustorial function of the lower end of the embryo sac.

9. As the seed develops the endosperm tissue is completely used up by the embryo. The structure of the mature seed is described in the paper.

10. The affinities of the Thymelaeaceae are considered, and it has been pointed out that, as Joshi (1936) suggests, the family is derived from dichlamydeous ancestors.

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