EMBRYOLOGICAL STUDIES IN THE LYTHRACEÆ.—III.

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Received March 9, 1936.
[Communicated by Prof. Y. Bharadwaja, M.Sc., Ph.D. (Lond.), F.L.S.]

This last part of these "Studies" deals with the structure and development of the ovule and the embryo-sac in *Ammania, Nesæa* and *Woodfordia*, and the development of pollen, male gametophyte, endosperm, embryo and seed in the family. At the end a summary is given of the chief embryological features of the Lythraceæ and a comparison is made with those of related families. The plants studied include, besides *Lawsonia inermis* and species of *Lagerstrœmia* described in the earlier papers, four species of *Ammania* (*A. baccifera* Linn., *A. multiflora* Roxb., *A. pentandra* Roxb. and *A. peploides* Spreng.), *Nesæa myrtifolia* Desf. and *Woodfordia floribunda* Salisb. Out of the four species of *Ammania*, two (*A. baccifera* and *A. multiflora*) belong to the sub-genus *Eu-Ammania* and the other two (*A. pentandra* and *A. peploides*) belong to the sub-genus *Rotala*. The material of all the above species of *Ammania* was collected locally from wild plants and was fixed either in Allen's modified Bouin's fluid or in Navaschin's fluid. Material of *Nesæa myrtifolia* was taken from a plant cultivated in the Royal Botanical Gardens, Sibpur, Calcutta, and was fixed in Karpetschenko's fluid. Material of *Woodfordia floribunda* was partly obtained from a plant grown at the Royal Botanical Gardens and partly from wild plants growing in the Kumaon Hills, Himalayas, during the course of a botanical excursion in those hills. It was fixed in both cases in Allen's modified Bouin's fluid. Heidenhain's iron-alum-hæmatoxylin was mostly used for staining the sections.

I. *Structure and Development of the Ovule and the Embryo-Sac in* Ammania, Nesæa *and* Woodfordia.

This account is based on a study of all the plants mentioned above except *Ammania peploides*. It thus includes three species of *Ammania*, *Nesæa myrtifolia* and *Woodfordia floribunda*. Two species of *Ammania* (*A. baccifera* and *A. pentandra*) and *Nesæa myrtifolia* are fully illustrated in the paper. Of *Ammania multiflora* only a few important stages are sketched, as it largely resembles *A. baccifera*. *Woodfordia floribunda* also is not fully illustrated here, as some observations on this plant have already been published by us elsewhere.[7] Here only those points are mentioned which were not at all or only inadequately described before.

Development of the inflorescence.—The development of the inflorescence has been studied in species of *Ammania* and fig. 1 clearly shows that the flower clusters in the axils of the leaves of *Ammania baccifera* develop in a cymose manner. The main axis is seen to end in the flower marked F_1 and a small flower F_2 is seen to arise from one side of it. In *A. pentandra* the flowers generally occur singly in the axil of the leaves, but sometimes a cymule of three also has been observed by us.

The gynæcium.—The placentation is marginal and axile in all the species except *Ammania baccifera*. In the latter species, the placentation is free central (figs. 2 and 3), but a column of tissue projects vertically inwards from the base of the style and closely approximates the apex of the free central placenta. To the naked eye or under a low magnification, the two may appear to be continuous. For this reason in the various taxonomic works and the Indian Floras the placentation is described to be axile, as it is in the family Lythraceæ in general, but microtome sections when examined under the microscope always show that the central placenta is quite free from the internal projection from the base of the style.

Although the placentation in *Ammania baccifera* is clearly free central, transverse sections commonly—we cannot say if always—reveal the presence of rudimentary septa of very short height just at the base of the ovary (fig. 5). There are 3 or 2 of these. When there are three, these are equally distributed. When there are only two, they are seen towards one side, as in fig. 5, clearly leaving the space for the third. This shows that the ovary in this species is tri-carpellary.

Fig. 4 illustrates an abnormal form of the free central placenta seen in *Ammania baccifera*. It was found to be much dichotomously divided, recalling to some extent the appearance of an abnormal case described by P. C. Joshi[10] in *Thylacospermum rupifragum*.

The style is bent upon itself in the bud stage in *Nesæa* just as in *Lawsonia*[8]. This condition is also seen in *Lagerstrœmia*, though it was not mentioned in the earlier paper.

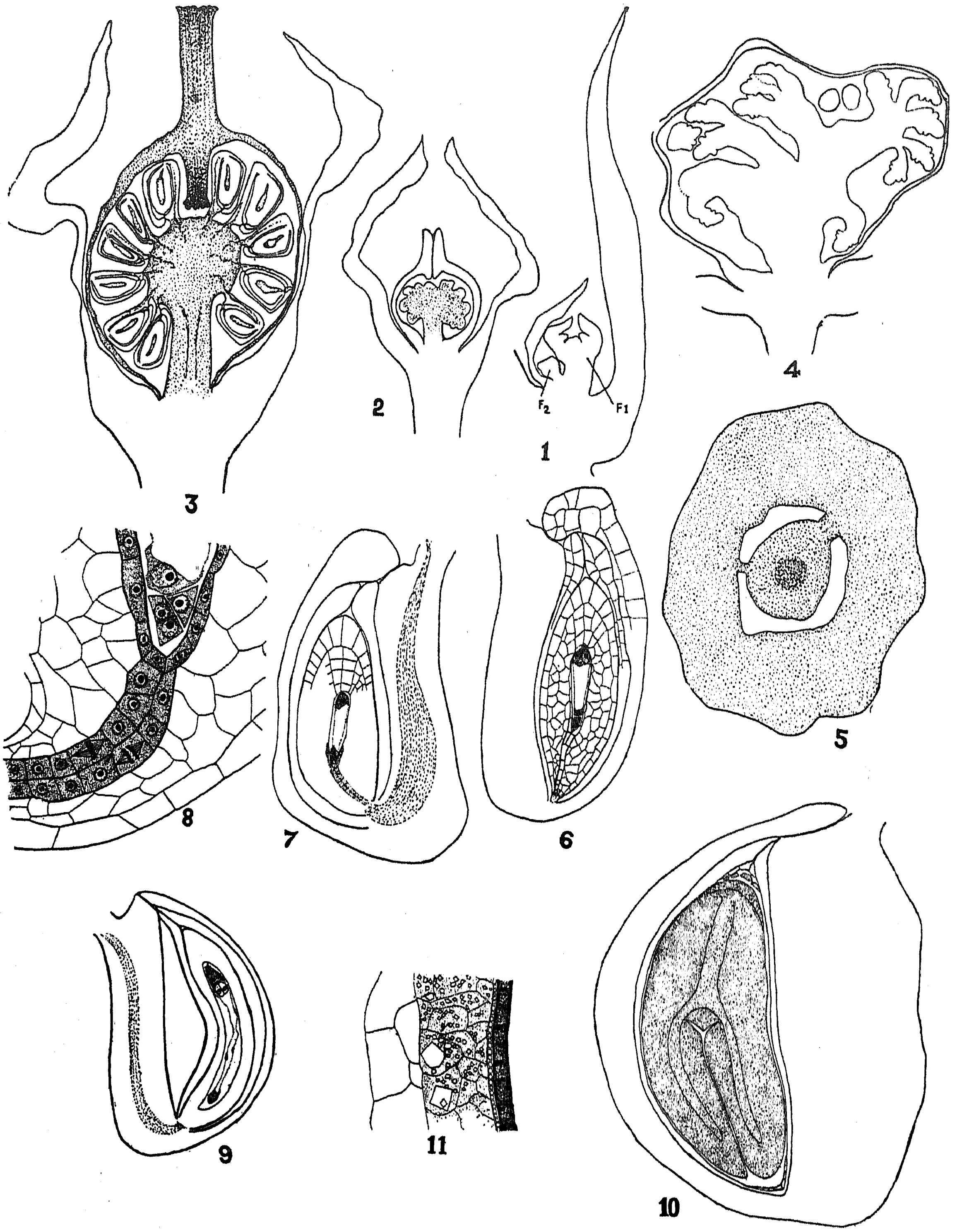
The ovule.—The ovules in every case are anatropous with the micropyle pointing downwards and possess two integuments. In *Nesæa myrtifolia*, however, the ovules near the base and the apex of the ovary are frequently amphitropous (figs. 51 and 52) and occasionally the micropyle of the ovules just at the base of the ovary points upwards (fig. 51). In one instance, an ovule was seen to be in an orthotropous condition at the megaspore-mother cell stage, when all other ovules of the ovary had assumed the anatropous form (fig. 50). This ovule also had, as an exceptional case, only a single integument. For a record of similar variations in other plants reference may be made to a paper by one of us published elsewhere (Joshi[5]). The ovules in all the forms show a slight flattening on the side of the raphe (fig. 7), especially near the apex, foreshadowing the large wing developed in seeds of *Lagerstrœmia*.

Both the integuments take part in the formation of the micropyle, as in *Lawsonia*[8] and *Lagerstrœmia*[9]. The portion of the micropyle formed by the outer integument is not in line with that formed by the inner integument (figs. 6 and 7). This is also seen in the ovules of *Lawsonia* and *Lagerstrœmia*, though this point was not noted in the earlier papers. Thus the micropyle in all Lythraceæ appears to be somewhat zig-zag, as described by Samal[12] in *Crotalaria juncea*. During the flowering stage in all the three genera—*Ammania*, *Nesæa* and *Woodfordia*—the integuments are mostly two cells, or here and there 3 cells, thick and consist of uniform cells. Their structure in the seed is described afterwards.

The nucellus in the fertilisable ovules consists of about 5 layers of cells above the embryo-sac, about 3 or 4 layers of cells on the sides of the embryo-sac and of about 7–10 layers of cells below the embryo-sac. It shows a slight curve in the chalazal region as in *Lawsonia* and *Lagerstrœmia* and a distinct strand of regularly arranged cells (figs. 7 and 8), connecting the vascular supply ending in the chalaza of the ovule with the base of the embryo-sac, just as in those genera.

Double nucelli.—Two nucelli within one ovule have been seen in *Nesæa myrtifolia*, just as in *Lagerstrœmia indica*[9] and in species of *Cuphea*[11]. In one case (fig. 53) both the outer and the inner integuments were common. In the second example (fig. 54), the outer integument was common to both the nucelli, but each had a separate inner integument of its own.

Megasporogenesis.—Our observations on the primary archesporium in the ovules of *Ammania*, *Nesæa* and *Woodfordia* agree with those made on *Lawsonia*[8] and *Lagerstrœmia*[9]. The primary archesporial cells are always many in number (figs. 12, 13, 14, 31, 55 and 66). While generally these cells are hypodermal, sub-hypodermal archesporial cells are quite common and have been found in every species investigated (figs. 12 and 66). A single archesporial cell has been exceptionally seen only in *Ammania pentandra* (fig. 32).

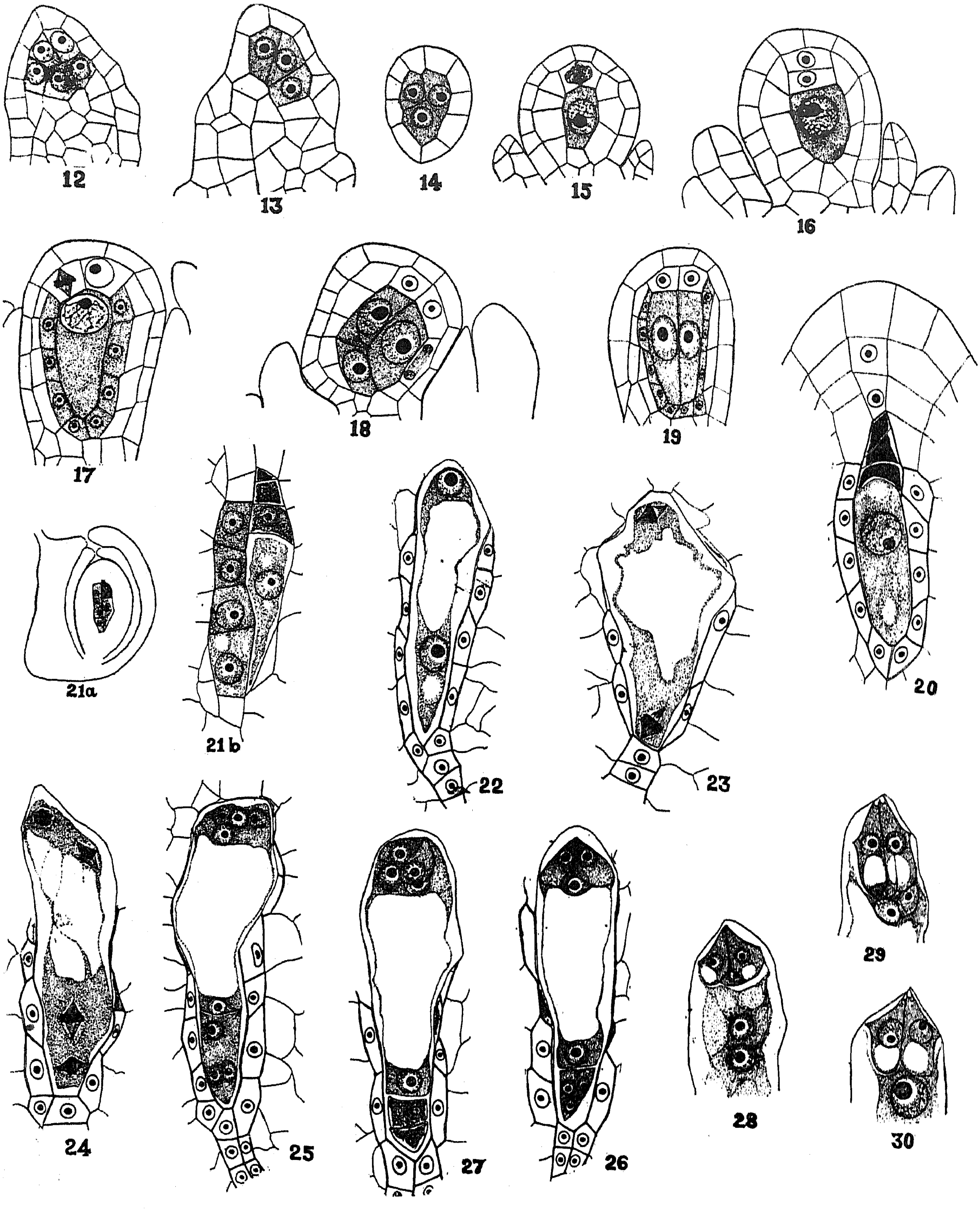


Figs. 1–11.—*Ammania baccifera.* Figs. 1-3, various stages in the development of the flower, especially gynæcium. Fig. 1 also shows the development of the inflorescence. The older flower (F_1) is terminal and the younger one (F_2) is seen arising from one side. Fig. 3 is a longitudinal section from an ovary containing nearly mature ovules and shows the free

central nature of the placentation. Fig. 4, longitudinal section of an ovary showing an abnormal much-divided free central placenta. Fig. 5, transverse section of an ovary near the base showing two septa. Figs. 6 and 7, longitudinal sections of ovules showing the form, micropyle, nucellus and the chalazal strand of conducting cells; fig. 7 along the raphe; fig. 6, a little towards one side of it. Fig. 8, the chalazal strand of conducting cells at a higher magnification. Figs. 9-10, two stages in the development of the seed; fig. 9 showing an embryo at the quadrant stage; fig. 10 showing a nearly mature embryo. Fig. 11, a portion of the testa from a nearly mature seed. Figs. 1, 2 and 4, × 260; Fig. 3, × 55; Fig. 5, × 90; Figs. 6 and 7, × 260; Figs. 9-10, × 125; Fig. 8, × 820; Fig. 11, × 315.

As in other plants of the family, one archesporial cell only cuts off a parietal cell and develops into the megaspore-mother cell and the other archesporial cells gradually lose their distinctive features and merge into the ordinary cells of the nucellus, but exceptions to this condition are very common and are seen in every species. Fig. 19 shows a case of two megaspore-mother cells in an ovule of *Ammania baccifera* lying side by side. These have developed from two adjacent archesporial cells after cutting off the parietal cells. Fig. 18 shows an ovule from the same plant with three sporogenous cells, two having taken origin from hypodermal archesporial cells and having cut off parietal cells, while the third has developed from a sub-hypodermal archesporial cell and has not cut off any parietal cell. Figs. 21*a* and *b* again illustrate from the same species an ovule in which two megasporocytes have even formed complete tetrads of megaspores. Figs. 34 and 36 illustrate two examples of double megaspore-mother cells from *Ammania pentandra*. In fig. 34, one of them has taken its origin from a hypodermal archesporial cell after cutting off a wall cell and the other from a sub-hypodermal archesporial cell and without cutting off a wall cell. The first is seen lying above the latter and both are in synizesis. In fig. 36, both the megaspore-mother cells are of hypodermal origin and have cut off parietal cells and one of them (on the left side in the figure) has already developed into a tetrad of megaspores. Fig. 44 shows an example of double megasporocytes developed from hypodermal archesporial cells after cutting off wall cells from *Ammania multiflora*. Fig. 58 shows the nucellus of an ovule from *Nesæa myrtifolia* with two megaspore-mother cells in synizesis, and lastly fig. 70 provides us with an example from *Woodfordia floribunda* with two megaspore-mother cells in nearly the same condition as described for *Ammania pentandra* in fig. 36.

The first division in the primary wall cell may be either anticlinal (figs. 15, 17, 34, 43 and 44) or periclinal (figs. 16, 36 and 57). This is followed by a number of divisions in all planes and ultimately results in the formation of 4 or 5 layers of parietal tissue above the micropylar end of the embryo-sac. A tapetum around the functioning megaspore-mother cell (or cells) is seen in species of *Ammania* (figs. 17, 19, 20), particularly in *Ammania baccifera*,



Figs. 12-30.—*Ammania baccifera.* Figs. 12-14, primary archesporium; in transverse section in fig. 14. Figs. 15-17, various stages in the development of the megaspore-mother cell and the parietal tissue. Fig. 18, three megaspore-mother cells in one ovule. Fig. 19, two megaspore-mother cells in one ovule. Fig. 20, a linear tetrad of megaspores. Fig. 21*a*, an

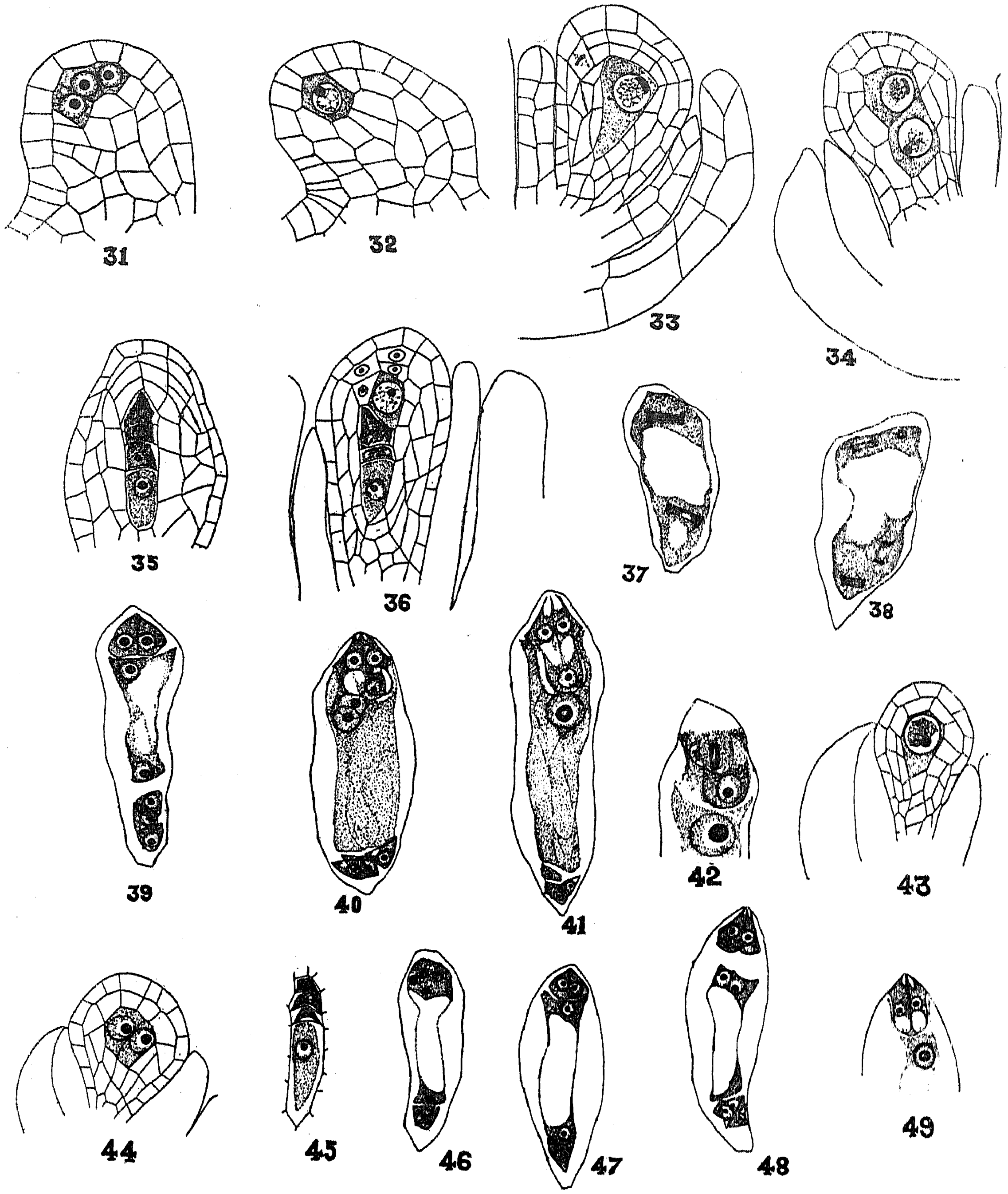
ovule with two tetrads of megaspores; fig. 21*b*, tetrads from the same at a higher magnification. Figs. 22-27, various stages in the development of the embryo-sac. Figs. 28-30, micropylar portions of the embryo-sac showing progressive stages in the differentiation of the egg-apparatus, particularly synergids and the secondary nucleus. All figs. except 21*a*, × 800; 21*a*, × 165.

but it is crushed during the development of the embryo-sac. The degeneration proceeds from the micropylar end towards the chalazal end, as the embryo-sac increases in diameter more in the first region. In other genera, such a layer of tapetal cells is only faintly differentiated from the other nucellar cells. Below the functioning megaspore-mother cell a row of axial cells is formed just as in *Lawsonia* and *Lagerstrœmia*. These cells by their division ultimately give rise to the strand of regularly arranged cells connecting the chalazal end of the embryo-sac with the vascular bundle of the ovule.

The functioning megaspore-mother cell undergoes a long period of rest and increases considerably in size before it undergoes the two meiotic divisions. In every case it gives rise to a complete tetrad of megaspores which has generally a linear form (figs. 20, 21*a* and *b*, 35, 36, 45, 60, 68 and 70), but may be occasionally in *Nesæa* and *Woodfordia* T-shaped (figs. 61 and 69). Development of the tetrad has been seen by us in *Nesæa* and *Woodfordia* and it appears that the chalazal dyad cell generally divides before the micropylar dyad cell (figs. 59, 67 and 68). The megaspore which develops into the embryo-sac is always the chalazal one and the other three in these plants have always been seen to degenerate.

Development and structure of the embryo-sac.—Development of the embryo-sac from the functional megaspore takes place in the normal manner (figs. 22-26, 35-40 and 62-63), except that the bi-nucleate embryo-sac, as in *Lagerstrœmia*, is often characterised by the persistence of the chalazal vacuole for an unusually long period (fig. 22) and sometimes this condition is seen even during the development of the 4-nucleate embryo-sac (fig. 37) or after its formation (fig. 62).

The antipodals are the first structures to differentiate in the embryo-sac (figs. 25 and 46). They generally show small or large vacuoles. In *Nesæa myrtifolia* and *Woodfordia floribunda*, as in other investigated Lythraceæ, they begin to degenerate very early and generally disappear by the time the polar nuclei fuse together (fig. 64). In species of *Ammania*, they have been found to be a little more persistent than in other members of the family and are often present in the embryo-sac up to the time of the formation of the secondary nucleus (fig. 41). Sometimes, however, even in this genus, they may degenerate very early (fig. 27). The polar nuclei which are at



Figs. 31-42.—*Ammania pentandra*. Figs. 31 and 32, primary archesporium. Fig. 33, megaspore-mother cell in synizesis. Fig. 34, two megaspore-mother cells in synizesis. Fig. 35, a tetrad of megaspores. Fig. 36, a tetrad of megaspores and a megaspore-mother cell lying in the same ovule. Figs. 37-41, various stages in the development of the embryo-sac. Fig. 42, micropylar portion of an embryo-sac at the time of fertilisation; a pollen-tube is seen with the generative nucleus in telophase. × 635.

Figs. 43-49.—*Ammania multiflora*; a few stages in the development of the embryo-sac. Fig. 43, megaspore-mother cell in synizesis. Fig. 44, two megaspore-mother cells. Fig. 45, a tetrad of megaspores. Figs. 46-48, three stages in the differentiation of the 8-nucleate embryo-sac. Fig. 49, mature synergids and the secondary nucleus. × 635.

first at either pole of the embryo-sac (figs. 26, 27, 39, 46 and 47) meet somewhere above the middle of the embryo-sac (fig. 28) and then move up to a position just below the egg-apparatus (figs. 29, 40, 48 and 63). Here they fuse and give rise to a large secondary nucleus before the pollen-tube enters the embryo-sac (figs. 30, 41, 49 and 64). During the development of the egg-apparatus, the synergids differentiate before the egg cell (figs. 26 and 27) and their nuclei are derived from a common mother nucleus. The nucleus of the egg and the upper polar nucleus form the second pair of sister nuclei, as in angiosperms in general. Our further observations on the structure of the egg-apparatus (figs. 28-30, 39-41, 47-49 and 63-65) agree with those made previously on *Lawsonia*^{8} and *Lagerstrœmia*^{9}. The mature synergids have always got prominent hooks and, besides the large chalazal vacuole, show a small vacuole in their apex as well.*

The form and size of the mature embryo-sac in *Woodfordia floribunda* has already been described. In species of *Ammania*, it is about 50-60μ long and is comparatively broader at the micropylar end and narrower towards the chalazal end. In *Nesæa myrtifolia*, the embryo-sac at the time of fertilisation measures about 80-100μ and is nearly equally broad at both the ends (fig. 65).

Degenerations in the embryo-sac, which are so commonly seen in *Lawsonia inermis*^{8} and species of *Lagerstrœmia*^{9}, are also observed in *Woodfordia floribunda*, but are absent in species of *Ammania*.

A case of reversed polarity in the embryo-sac was seen by us in *Woodfordia floribunda* and has already been described^7.

II. Development of Pollen and Male Gametophyte.

The development of pollen and male gametophyte has been followed in two species of *Ammania* (namely, *A. baccifera* and *A. pentandra*), *Lawsonia inermis* and *Nesæa myrtifolia* in all its stages, except the differentiation of primary archesporium, which has been observed only in preparations from *Ammania*. In the two species of this genus, the primary archesporium consists in each of the four lobes of the anther of a hypodermal row of 6 to 9 cells, as may be judged from longitudinal (fig. 72) and transverse (fig. 73) sections of the young anthers. The first division in the primary archesporial cells is

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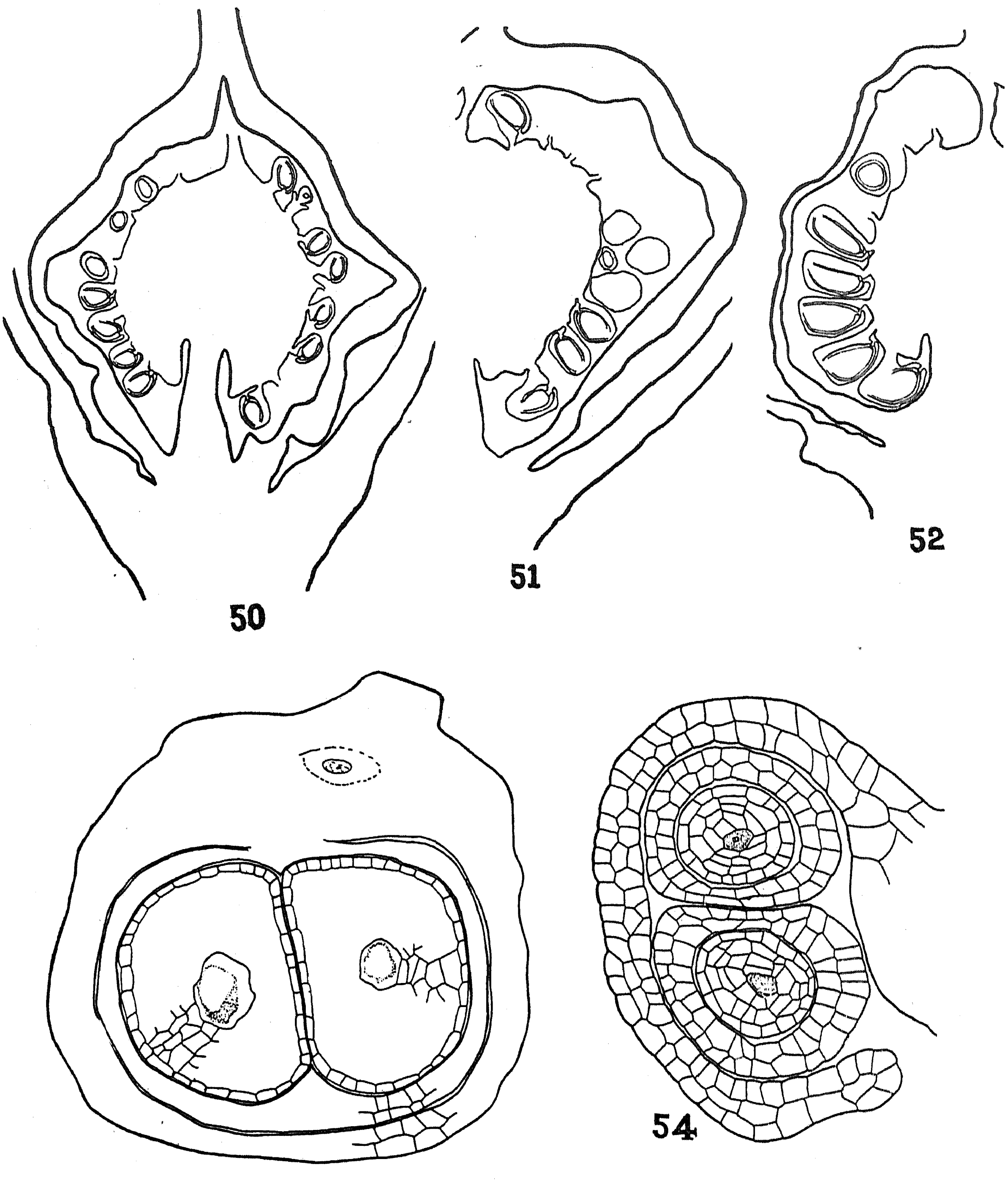
* Some previous observations published by us on the synergids of *Ammania baccifera* (6) are incorrect. These had resulted from a confusion of the synergids with the strong accumulation of endosperm (described later) formed in the micropylar part of the embryo-sac, which was caused by the absence of certain developmental stages in the material then examined and the presence of pollen-tubes in the embryo-sac even after the development of the endosperm had begun.



Figs. 50–54.—*Nesœa myrtifolia.* Figs. 50–52, longitudinal section (fig. 50) or portions of longitudinal sections (figs. 52–53) of the ovary showing placentation and the form of the ovules. Fig. 53, two nucelli within common outer and inner integuments. Fig. 54, two nucelli within a common outer, but separate inner integuments. Figs. 51–52, × 45; Fig. 53, × 375; Fig. 54, × 440.

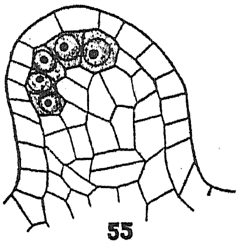
periclinal as usual (figs. 72 and 74) and leads to the formation of the layer of primary parietal cells towards the outside and primary sporogenous cells towards the inside. The first periclinal division in the primary parietal cells gives rise to two layers (fig. 74). The cells of the outer one of these do not generally divide any more periclinally, but those of the inner divide once more in a periclinal fashion and thus give rise to two layers of narrow flattened cells (figs. 75 and 76). In all the genera in this manner three layers of parietal cells are formed below the epidermis. The primary sporogenous cells undergo a number of divisions in all planes and give rise to a fairly large amount of sporogenous tissue.

As figs. 75 and 76 show, the tapetum seems to be derived largely from the outer sporogenous cells. Through its greater part it consists of a single layer of cells, but here and there it is even 2-seriate (fig. 76, at the points marked *t*). About the time that the microsporocytes enter synizesis, the nuclei of the tapetal cells undergo an ordinary mitotic division, just as Cooper[1] has recently demonstrated in so many other angiosperms, and become 2-nucleate. Most of the tapetal cells remain in this condition throughout their life, but the nuclei in some tapetal cells undergo further divisions, most probably of the amitotic type, and these tapetal cells become 3-6-nucleate.

The microspore-mother cells do not separate from one another and round off prior to the two meiotic divisions, but remain packed together (fig. 76). The meiotic divisions take place in the normal manner. At the end of the first meiotic division, an evanescent cell-plate is formed. At the end of the homotypic division, the grand-daughter nuclei become connected with one another by means of secondary spindle fibres. Cytokinesis takes place by furrowing. Pollen grains are formed in a simultaneous manner. They are generally arranged in a tetrahedral manner (fig. 81), but bilateral arrangement is also frequent.

During the formation of the pollen grains, the anthers undergo the usual increase in size and the epidermis of the anther-lobes and the layer of parietal cells just below the epidermis, which later on develops into the fibrous endothecium, keep pace with this growth by means of anticlinal divisions. The two layers of parietal cells between the fibrous endothecial layer and the tapetum are crushed early due to the growth of the latter. The tapetal cells begin to degenerate after the formation of tetrads and ultimately disappear. Here and there a tapetal cell may be seen lying in between the young pollen grains or tetrads, but no periplasmodium is formed.

The pollen grains, as usual after tetrad formation, separate and round off before the differentiation of exine and intine begins, but in one case in *Nesæa myrtifolia* they had failed to separate even when they had undergone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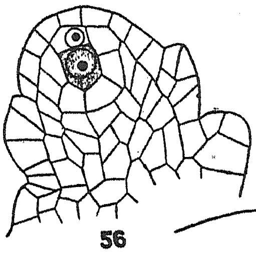
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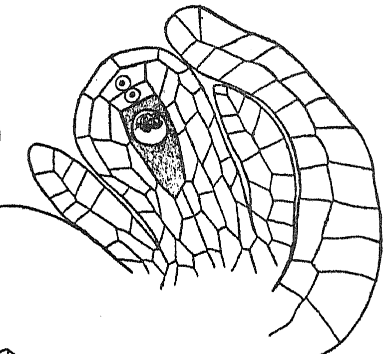
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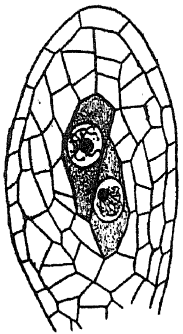
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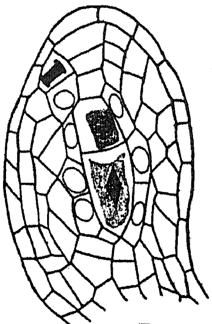
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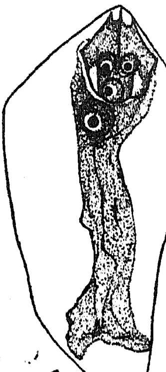
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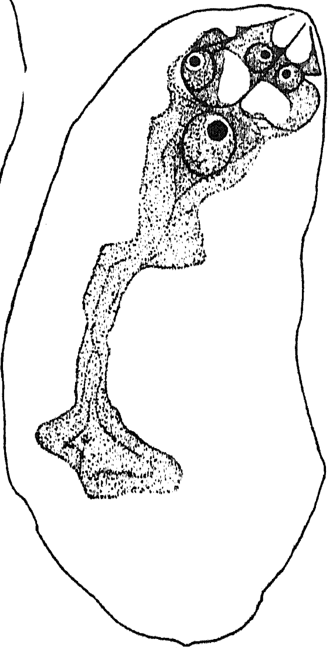
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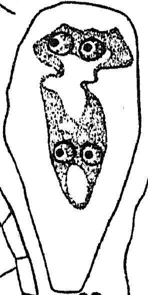
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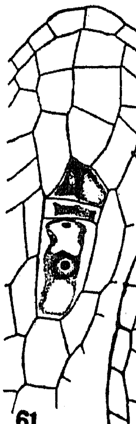
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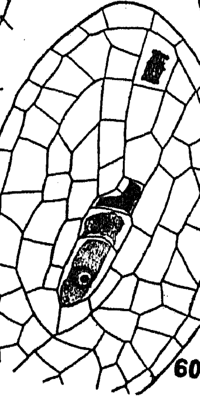
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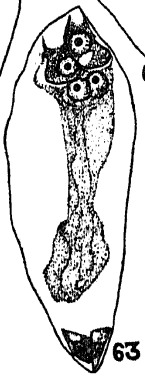
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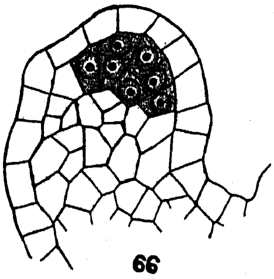
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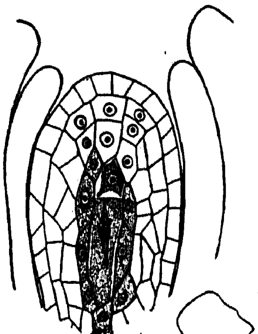
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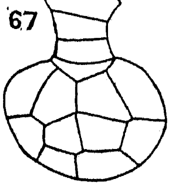
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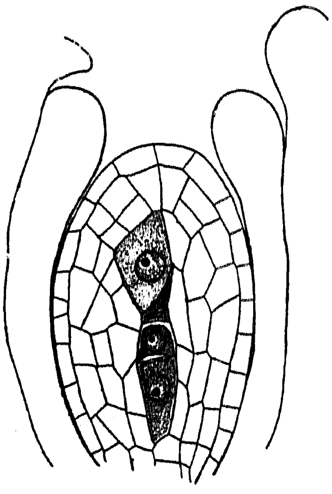
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Figs. 55-65.—*Nesæa myrtifolia.* Figs. 55–56, primary archesporium and the cutting off of the primary wall cell. Fig. 57, megaspore-mother cell in synizesis. Fig. 58, a nucellus with two megaspore-mother cells. Fig. 59, nucellus showing development of the tetrad of megaspores. Fig. 60, a nucellus with a linear tetrad of megaspores. Fig. 61, a T-shaped tetrad of megaspores. Fig. 62, 4-nucleate embryo-sac; the chalazal vacuole is still present. Fig. 63, a young 8-nucleate embryo-sac. Figs. 64–65, older embryo-sacs, after the degeneration of the antipodals. × 600.

Figs. 66–71.—*Woodfordia floribunda.* Fig. 66, primary archesporium. Figs. 67–68, two stages in the development of the tetrad of megaspores. Fig. 69, a T-shaped tetrad of megaspores. Fig. 70, an ovule with one tetrad of megaspores and a megaspore-mother cell. Fig. 71, a stage in the development of the embryo. × 600.

their full development (fig. 81). The first division of the nucleus in the pollen grains (fig. 77) takes place after the exine and intine have become fully differentiated and the pollen grains have undergone most of their development in size, and is followed by the formation of a cell-plate and a lenticular generative cell is formed separated by a curved wall from the vegetative cell (figs. 78 and 81). Later on, the intervening wall between the two nuclei disappears and the pollen grains are in this 2-nucleate condition at the time of shedding (figs. 79 and 80). The division of the generative nucleus to form two male nuclei takes place just before fertilisation, at the apex of the pollen-tube, as it reaches the embryo-sac (fig. 42).

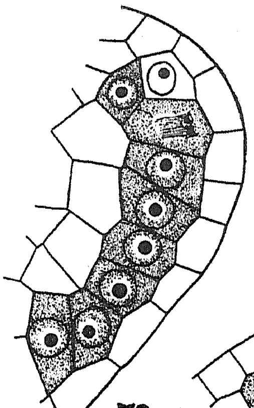
In form the mature pollen grains are nearly globose, but in *Ammania baccifera* they are slightly elongated, so that one diameter is slightly longer than the other at right angles to it (figs. 77-80). Sections of pollen grains along these different diameters, therefore, give different appearances and one may at first be wrongly led to believe that the pollen grains are dimorphic. There are generally 3 germ pores in the exine of each pollen grain arranged in an equitorial fashion (figs. 77-80).

Degenerations in the sporogenous tissue in the anthers are quite common both in *Nesæa* and *Lawsonia* and are also met with in species of *Ammania.* They occur during all stages of development,—in the primary archesporial cells (fig. 74), in the microspore-mother cells, in one, two, three (figs. 82 and 83) or all the pollen grains of a tetrad while inside the mother-cell wall, and in 1- and 2-nucleate nearly mature pollen grains. They may affect only a few cells of the loculus of an anther, but sometimes the whole loculus may be affected (fig. 84).

III. Fertilisation, Endosperm and Development of Embryo and Seed.

The above-mentioned phases of the life-history have been studied fully in the genus *Ammania,* particularly in two species *A. baccifera* and *A. pentandra,* and to some extent in *Woodfordia floribunda.*

Fertilisation.—The pollen-tube enters the ovule by way of the micropyle and fertilisation is porogamous. Some pollen-tubes have been seen in the



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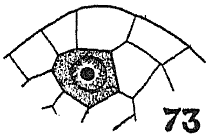

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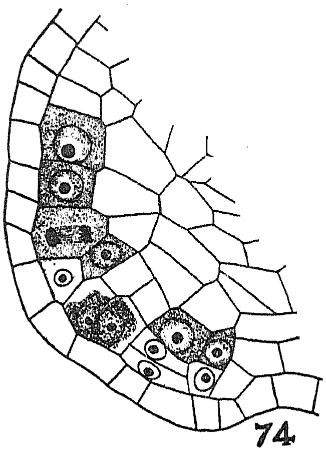

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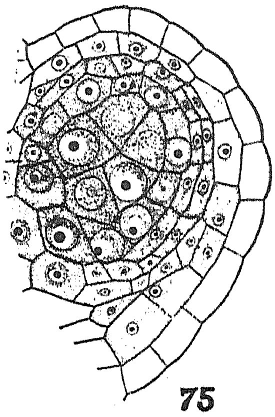

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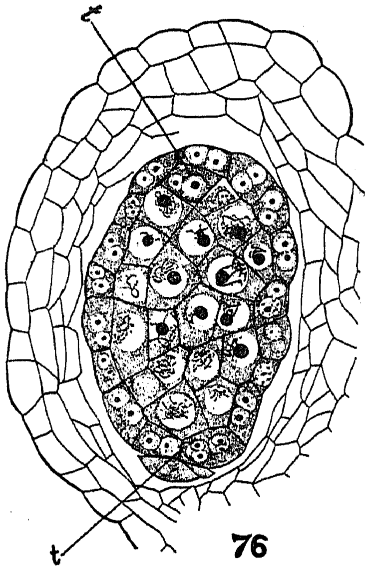

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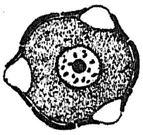

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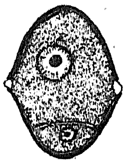

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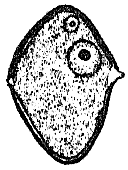

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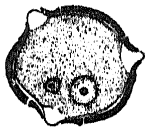

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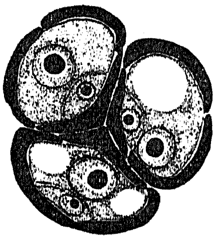

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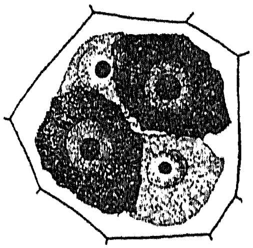

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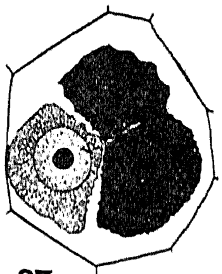

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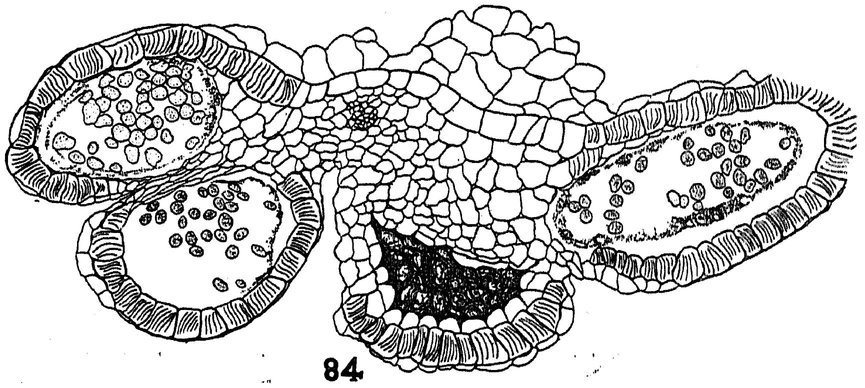

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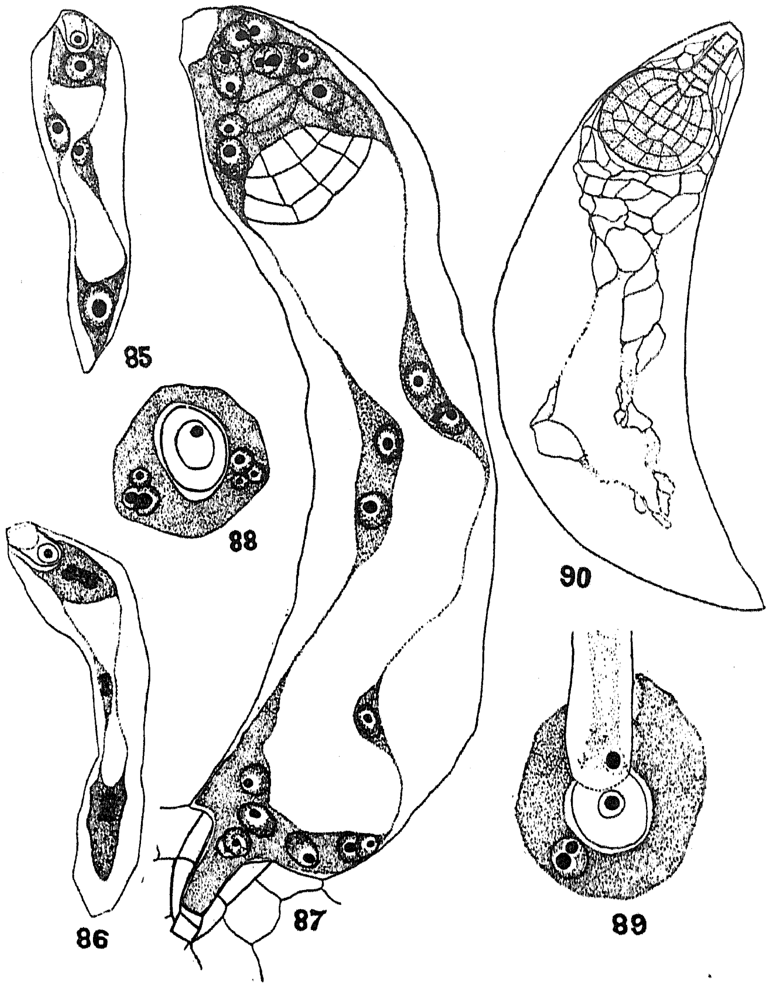
Figs. 72–73, *Ammania baccifera,* longitudinal and transverse sections respectively of two anther-lobes showing the primary archesporium. Fig. 74, *Ammania pentandra,* longitudinal section of an anther-lobe, showing two primary archesporial cells at the upper end, next a cell showing the first division of the primary archesporium, next a primary archesporial cell which has already divided into the primary wall cell and the primary sporogenous cell, next two degenerating primary archesporial cells, next the first periclinal division of the primary wall cell and lastly at the lower end a primary archesporial cell which has only completed the division into the primary wall and sporogenous cells. Fig. 75, *A. baccifera*, transverse section of an anther-lobe just after the differentiation of tapetum. Fig. 76, *A. baccifera*, transverse section of an anther-lobe with microspore-mother cells in synizesis. *t*, biseriate tapetum. Figs. 77-80, *A. baccifera*, pollen grains in various stages of development, in figs. 77 and 80 cut along the shorter diameter, in figs. 78 and 79 cut along the longer diameter. Fig. 81, *Nesæa myrtifolia*, an abnormal tetrad showing the full development of pollen grains without separating from one another. Figs. 82–83, *Lawsonia inermis*, pollen-mother cells showing degeneration of some young pollen grains while still inside the mother cell wall. Fig. 84, *Lawsonia inermis*, transverse section of an anther showing degeneration of one complete loculus. Figs. 72-73, × 940; Fig. 74, × 820; Figs. 75-76, × 480; Figs. 77-80, × 1440; Fig. 81, × 940; Figs. 82 and 83, × 1600; Fig. 84, × 145.

embryo-sac in *Ammania baccifera* even a long time after fertilisation and even when a good deal of endosperm has been formed (fig. 89). Double fertilisation has been observed in the same species.

Endosperm.—The formation of the endosperm begins before the first division of the oospore (figs. 85 and 86). Our observations on its development agree with those of Mauritzon[11]. The development takes place according to the nuclear type. After the first division of the primary endosperm nucleus, one of the daughter nuclei migrates to the chalazal end of the embryo-sac, while some daughter nuclei of the second reach the micropylar apex (fig. 85). An accumulation of cytoplasm now takes place at both the ends of the embryo-sac, while the protoplasmic lining on the sides of the embryo-sac remains very narrow. The nuclei that have reached the two ends of the embryo-sac divide very quickly by mitotic divisions (fig. 86) and soon accumulate in large numbers (figs. 87 and 88). In this manner chalazal and micropylar accumulations of endosperm develop. The micropylar accumulation of endosperm in *Ammania* is larger than the chalazal, just the reverse of what Mauritzon has described in *Peplis*. Fusions of nuclei in these accumulations of endosperm are commonly seen in later stages.

The endosperm remains free nuclear for a fairly long time, but ultimately becomes cellular as the three histogenic layers differentiate in the embryo (fig. 90). The large central vacuole, however, is never completely filled up with cells.

During the development of the endosperm, the embryo-sac undergoes great increase in length, and becomes slightly bent at both ends. At the chalazal end, in species of *Ammania*, a pouch-like structure is first formed, just as Mauritzon has figured in *Peplis portula*, piercing the chalazal strand of conducting cells (fig. 87), but after some time the end of the embryo-sac becomes even.



Figs. 85–90.—*Ammania baccifera*. Figs. 85–87 and 90, various stages in the development of the endosperm. Fig. 88, a transverse section of the micropylar accumulation of endosperm. Fig. 89, an oblique section of the micropylar accumulation of endosperm showing the presence of a pollen-tube even when a considerable amount of endosperm has been formed. Figs. 85–88, × 600; Fig. 89, × 770; Fig. 90, × 250.

*Development of embryo**.—The development of the embryo agrees with that of *Lythrum Salicaria* as described by Souéges[14], except in small details, and corresponds to the "*Capsella*-type". The first division of the oospore is transverse (figs. 91 and 108) and this is followed by another transverse division in the basal cell (fig. 92). In this manner a row of three cells is formed (figs. 93 and 109). In *A. baccifera*, longitudinal divisions in the

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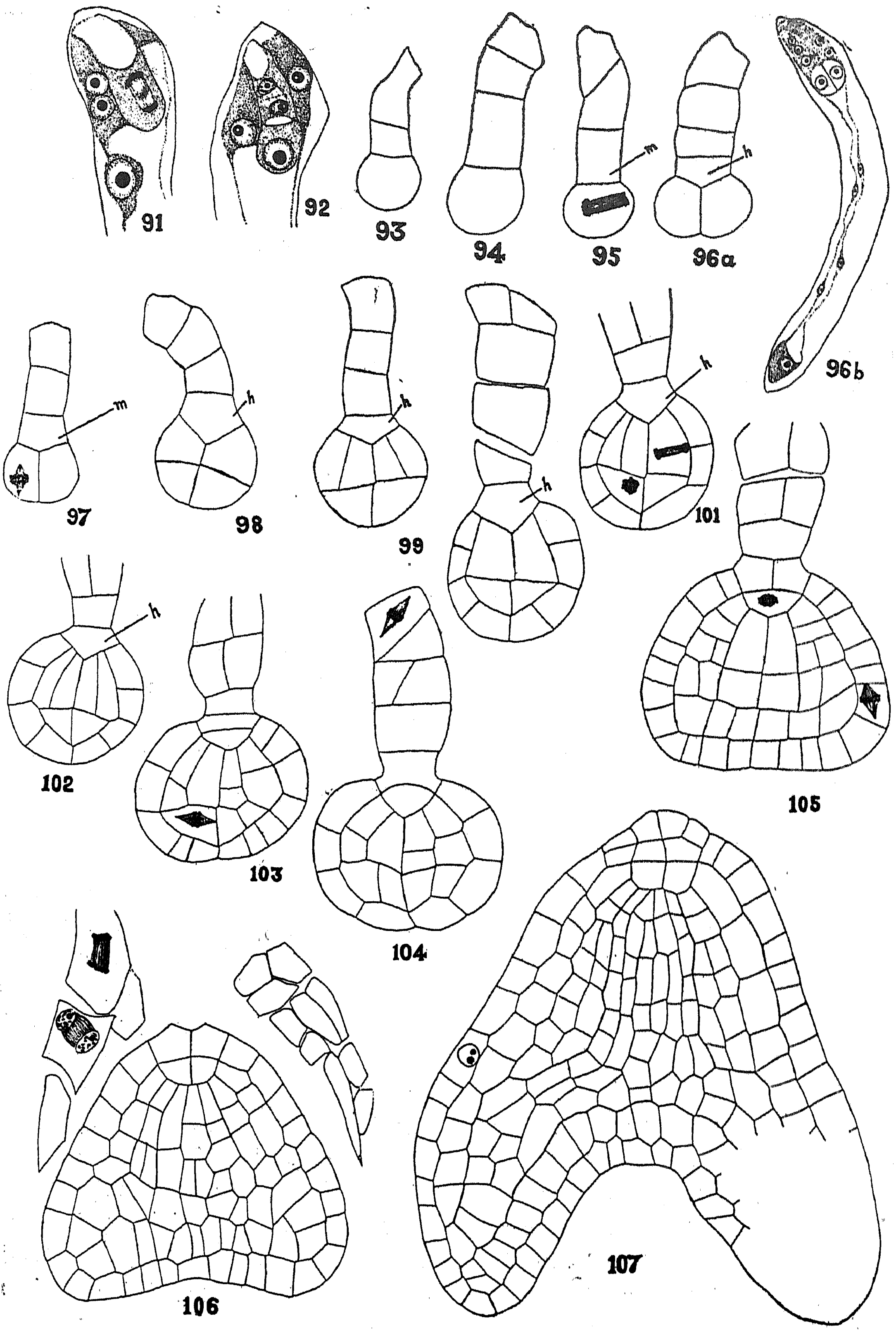
* The development of the embryo in species of *Ammania* is very easy to follow. Sometimes a single cluster of flowers in *Ammania baccifera* may yield all the important stages. As the material of this genus is found throughout India, it can be recommended as a suitable type for study by various classes in preference to *Capsella*, the material of which is available only in certain parts of the country.

apical cell appear after this stage (fig. 110). In *A. pentandra*, one more transverse division takes place in the inferior-most cell before the appearance of the longitudinal walls in the apical cell (figs. 94 and 95). Thus the proembryo in *A. baccifera* consists of a row of three cells, while in *A. pentandra* generally of a row of four cells. In *Lythrum Salicaria*, the longitudinal divisions in the apical cell take place after the first transverse division of the oospore and the proembryo consists of only two cells. This is however not a point of a great difference between the two genera. As Souèges has shown, transverse divisions in the basal cell of the proembryo in *Lythrum Salicaria* take place afterwards. The difference consists only in the fact that these divisions in species of *Ammania* take place earlier. The same feature is seen in *Peplis portula*, *Ammania senegalensis* and *Nesæa syphilitica* studied by Mauritzon[11], and in these plants also the proembryo consists of 3 cells.

The terminal cell of the proembryo from an early stage assumes a more or less spherical shape and undergoes two longitudinal divisions in planes at right angles to each other and gives rise to the quadrants (figs. 96 and 111). This is followed by transverse divisions in each of the quadrants (fig. 97) and the octants are formed (figs. 98 and 112). The four superior octants give rise to the cotyledons and the stem-tip, while the four inferior octants give rise to the hypocotyl and the primary root except its apex. The hypophysis (marked *h* in figures) differentiates rather early during the development of the embryo, just as the quadrants (fig. 96) or the octants (fig. 112) are formed. It arises from the superior daughter cell of the penultimate cell of the proembryo (marked *m* in figures) and gives rise to the apex of the root.

The differentiation of the three histogenic layers proceeds just as in *Capsella* (figs. 99-107 and 113-116). It takes place earlier in the inferior octants. In the portion formed from the superior octants, the differentiation of plerome takes place very late,—after the differentiation of the cotyledons has taken place (fig. 107). The stem-tip differentiates very late, after the cotyledons have undergone a great part of their development, and remains even in the mature embryo a very small structure (fig. 117). The hypophysis cell divides first transversely giving rise to two cells, one superposed upon the other (figs. 103-104 and 114-115). Each of these divides by longitudinal walls to form a plate of four cells (figs. 106-107 and 118). The divisions in the cell next to the suspensor take place before those in the superior cell (figs. 105 and 116). The plate of four superior cells formed from the hypophysis completes the periblem at the apex of the root, while the four inferior cells complete the dermatogen and the root-cap (figs. 106–107 and 116 and 118).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Figs. 91–107.—*Ammania pentandra.* Longitudinal sections of various stages in the development of embryo. In figs. 91 and 92 the micropylar part of the embryo-sac is also shown. Fig. 96*b* shows the whole embryo-sac containing an embryo at the stage sketched in fig. 96*a*. In fig. 106 some of the endosperm cells surrounding the embryo are also shown. *h*, hypophysis; *m*, mother cell of the hypophysis. Figs. 91-96*a*, × 630; Fig. 96*b*, × 300; Figs. 97-107, × 630.

The suspensor is formed from the cells of the proembryo not taking part in the formation of the embryo proper and the inferior daughter cell of the penultimate cell of the proembryo. Some of these cells undergo a few transverse divisions, so that the suspensor becomes 4 or 5 cells long. In *Ammania baccifera*, it remains uni-seriate throughout its development, but in *A. pentandra* longitudinal walls also appear in all the suspensor cells, except in the sister cell of the hypophysis, so that through its greater length the suspensor becomes more than 1-seriate. The structure of the suspensor in *A. multiflora* agrees with that of *A. baccifera*, and that of *A. peploides* with that of *A. pentandra.* As has been mentioned before, the first two species belong to the sub-genus *Eu-Ammania* and the latter two to *Rotala*. The embryological findings thus support the current classification.

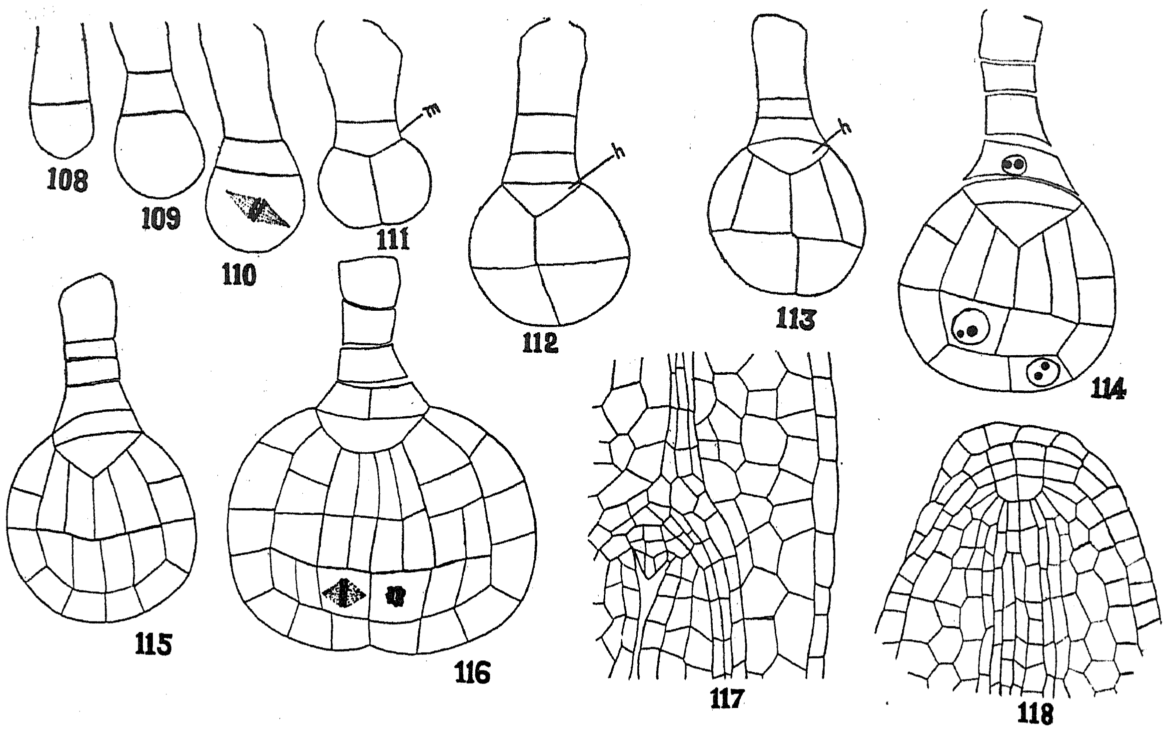
A few stages of embryo development have also been observed in *Woodfordia floribunda*, and as fig. 71 shows this agrees with the development in *Ammania* as described above.

Structure of the mature seed.—During its development, the embryo destroys the whole of the endosperm and later on the nucellus. The mature seed is thus exalbuminous (fig. 10). In the testa of the mature seed, the cells of inner layer of the inner integument become greatly thickened, while those of the outer layer do not undergo much change. Similarly, the cells of the outer layer of the outer integument also do not change very much, but those of the inner divide and give rise to about three layers of cells in which certain crystals are abundantly deposited (fig. 11).

During the development of the seed after fertilisation, the outer integument separates off from the inner on the side of the raphe. A large air-space thus appears in between the two (fig. 9). It has been seen in all species of *Ammania* mentioned above. The purpose of this space is probably to help in aeration during a period of great metabolic activity, as all these species grow in marshy places. During further growth of the seed, this air-space gradually disappears (fig. 10).

IV. Summary of the Embryological Features of the Lythraceæ.

The ovule.—The ovules in the Lythraceæ are generally anatropous and ascending. Only exceptionally they are found to be descending. In *Nesæa* only some of the ovules have been seen by us to have an amphitropous form.



Figs. 108-118.—*Ammania baccifera.* Figs. 108-116, longitudinal sections of various stages in the development of the embryo from the two-celled stage onwards. Fig. 117, a portion of the mature embryo showing the stem-tip and portions of cotyledons. Fig. 118, a portion of the mature embryo showing the root-apex. *h,* hypophysis; *m,* mother cell of hypophysis. × 780.

There are two integuments and both take part in the formation of the micropyle, which has been found to have in every case a somewhat zig-zag and not straight form.

The chief feature of the nucellus is the presence of a strand of regularly arranged, somewhat thick-walled cells in the chalazal region, connecting the vascular bundle of the ovule ending in the chalaza with the base of the embryo-sac. We have not performed any chemical tests to see if these cells are lignified, but there is little doubt that this strand corresponds to the hypostase described in the Onagraceæ[3] and other Myrtifloreæ. The probable function of this strand of cells is to conduct food materials to the embryo-sac.

The occurrence of two nucelli within one ovule seems to be quite common within the family. Mauritzon[11] has noted this condition in *Cuphea lanceolata* and *Cuphea petiolata.* We have noted this in *Lagerstrœmia indica*[9] and *Nesæa myrtifolia.* These double nucelli may have common outer and inner integuments, or the outer integument alone may be common, and each nucellus may have a separate inner integument.

The female archesporium.—The primary archesporium in the ovule in all the forms investigated by us has been found to be multicellular. A single archesporial cell has been seen only occasionally in the genus *Ammania.* Mauritzon[11], however, has described a single-celled primary archesporium as characteristic of the family. These observations to our mind appear to be based on inadequate study of the early stages.

That the multicellular primary archesporium is the general condition in the family is supported by the common occurrence of two or three megaspore-mother cells or tetrads of megaspores and in some cases, as in *Lawsonia* and *Lagerstrœmia,* even of more than one embryo-sacs in the same ovule. There is not a single species investigated by us in which we have not come across, and also in a fair number of cases, two or three archesporial cells cutting off the parietal cells and developing into megaspore-mother cells.

Among the allied families, according to Schnarf[13], a multicellular primary archesporium has been described in the Rhizophoraceæ (*Rhizophora Mangle, R. mucronata, Ceriops Candolleana* and *Bruguiera* species) by Karsten, Nyssaceæ (*Davidia involucrata*) by Horne, and a number of Onagraceæ (species of *Epilobium, Godetia, Oenothera,* etc.) by Michaelis, Schwemmle, Täckholm, Håkansson, etc. Recently in *Callistemon linearis* (Myrtaceæ), Tiwary and Rao[16] have also noted the occasional occurrence of multicellular primary archesporium. Some authors, however, doubt the presence of such multicellular archesporium. For instance, Johansen[4] writes, " I am constrained to regard with scepticism some of the published accounts of ' several archesporial cells ' in various onagrads." From what, however, we have found in the Lythraceæ, we think that these accounts are most probably correct and the occurrence of double or triple embryo-sacs in some Onagraceæ (Ishikawa[2]) supports such a conclusion.

Megasporogenesis.—The functional archesporial cells always cut off a parietal cell except when they are sub-hypodermal in position. The amount of parietal tissue formed varies in the different genera.

A linear tetrad of megaspores is commonly formed, but occasionally T-shaped tetrads also occur, as has been seen in *Lawsonia, Nesæa* and *Woodfordia.* During the development of the tetrad, often the micropylar dyad cell divides later than the chalazal. According to Mauritzon[11] in some forms (*Peplis portula, etc.*) the nuclear division in the upper dyad is not followed by the formation of a cell-wall, so that a 2-nucleate micropylar megaspore is formed, or sometimes even the nuclear division in the upper dyad is suppressed. We have, however, not come across any such case in the forms investigated by us.

The chalazal megaspore of the tetrad is always the functional one. Only exceptionally in *Lawsonia inermis*[8] we have seen the second megaspore from the chalazal end enlarging and sometimes developing into the embryo-sac. In no case the micropylar megaspore has been seen to develop into the embryo-sac, although this is the fixed condition in the Onagraceæ.

Embryo-sac.—The chief noteworthy feature in the development of the embryo-sac is the persistence of the chalazal vacuole during the 2- and 4-free-nucleate stages. This has been seen in many forms both by Mauritzon[11] and by us. Characteristic of the mature embryo-sac is the early degeneration of the antipodals. This has been seen in every species investigated by us and other recent authors. Both these points, besides a few other minor ones, have been regarded by Tischler[15] and Mauritzon[11] to indicate that the embryo-sac of the Lythraceæ forms phylogenetically an intermediate stage between the 4-nucleate embryo-sac of the Onagraceæ and the normal 8-nucleate embryo-sac. To these arguments we may now add the various stages in the reduction of the antipodal nuclei described by us in *Lawsonia inermis*[8] as forming a still stronger connecting link.

The structure of the synergids in the Lythraceæ is characteristic in that they always develop a prominent hook and in later stages a small vacuole in their apex besides the large chalazal vacuole. The apical vacuole takes the place of the 'filiform apparatus' seen in other plants and serves probably the same function, namely, that of secreting chemotropically active substances which guide the pollen-tube.

During the secondary elongation after fertilisation, or sometimes it may be even before fertilisation, of the embryo-sac, the antipodal end often grows either into a pouch-like structure penetrating the chalazal strand of conducting cells (*Ammania* and *Peplis*) or forms a postament around the chalazal strand due to the resistance offered by the latter (*Cuphea* and *Lagerstrœmia*)*.

Pollen and male gametophyte.—The primary archesporium in each lobe of the anther differentiates as a single row of hypodermal cells. Between the tapetum and the epidermis, typically three layers of cells are formed. The outer one of these forms the fibrous endothecium. The other two disorganise. Tapetal cells are 2-6-nucleate. They may have (*Lawsonia*, *Cuphea*, etc.) or may not have (*Ammania*, etc.) prominent vacuoles. Periplasmodium formation does not take place. A generative cell in the pollen grains is always formed at first, but later on the wall separating it from the

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* The latter feature is also seen in the Myrtaceæ (*Callistemon linearis*), as is evident from the figure of Tiwary and Rao.[16]

vegetative cell dissolves. The pollen grains are 2-nucleate at the time of shedding and possess three germ spores arranged in an equatorial plane. The division of the generative nucleus to form two male nuclei takes place rather late, just before fertilisation.

Fertilisation.—Fertilisation is porogamous. Double fertilisation has been seen in *Ammania*.

Endosperm.—The endosperm in the Lythraceæ develops in the free nuclear manner. It has been found to be scanty in all the investigated forms and in this respect the family agrees with the Onagraceæ. Micropylar and chalazal accumulations of endosperm are commonly formed during the early stages.

Embryogeny.—The development of the embryo takes place according to the "*Capsella*-type". The chief difference consists in the early differentiation of the hypophysis. The development differs from the Onagraceæ in the origin of the hypophysis. In Onagraceæ, the hypophysis is formed directly from the penultimate cell of the proembryo. In Lythraceæ, it is formed from the superior daughter cell of the penultimate cell of the proembryo.

Within the family Lythraceæ, the different forms vary in the form of the suspensor. The suspensor remains throughout uni-seriate in *Peplis*, *Nesæa* and some species of *Ammania* belonging to the sub-genus *Eu-Ammania*. It has been found to become multi-seriate in the genus *Cuphea* and some species of *Ammania* belonging to the sub-genus *Rotala*.

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