EMBRYOLOGICAL STUDIES IN THE LYTHRACEAE.

II. *Lagerstrœmia* Linn.

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The present paper describes the structure and development of the ovule and embryo-sac in two species of *Lagerstrœmia*, namely, *L. indica* Linn. and *L. Flos-Reginæ* Retz. The material of these was collected from plants cultivated in the Benares Hindu University grounds and was prepared for study according to the customary methods. It was fixed in Allen's modified Bouin's fluid and Heidenhain's Iron-alum-hæmatoxylin was used for staining the sections.

Structure of the Ovary.

The ovary in the genus *Lagerstrœmia* is generally described to be 3–6 celled, but during the present investigation it has been occasionally seen to be 7-celled in *L. Flos-Reginæ* (Fig. 1). Further in both the species, the midribs of the carpels, which alternate with the septa, protrude inwards into the cavity of each loculus forming a kind of secondary septa. These reach up to about the middle of each loculus of the ovary and divide it partially into two halves. The ovules which are very large in number are borne in an axile manner and there are four rows of them in each loculus, two in each half (Fig. 1).

The Ovule and the Nucellus.

The ovules are anatropous and ascending. Those of *Lagerstrœmia indica* about the time of fertilisation measure about 550 *u* in length, while those of *L. Flos-Reginæ* about the same time are 800–900 *u* long. Their chalazal end is flattened towards the side of the raphe. The ovules therefore when cut longitudinally along this plane appear triangular (Fig. 31 *a*). When cut in a plane at right angles to this, these appear nearly cylindrical (Fig. 2). A transverse section of the ovule near the micropylar end is nearly circular, while a transverse section from near the summit of the ovule shows a marked narrow prolongation towards one side (Fig. 3). This flattened summit of the ovule develops in the mature seed into a prominent wing.

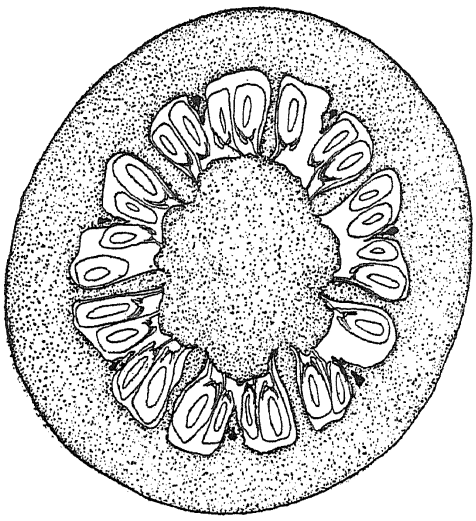


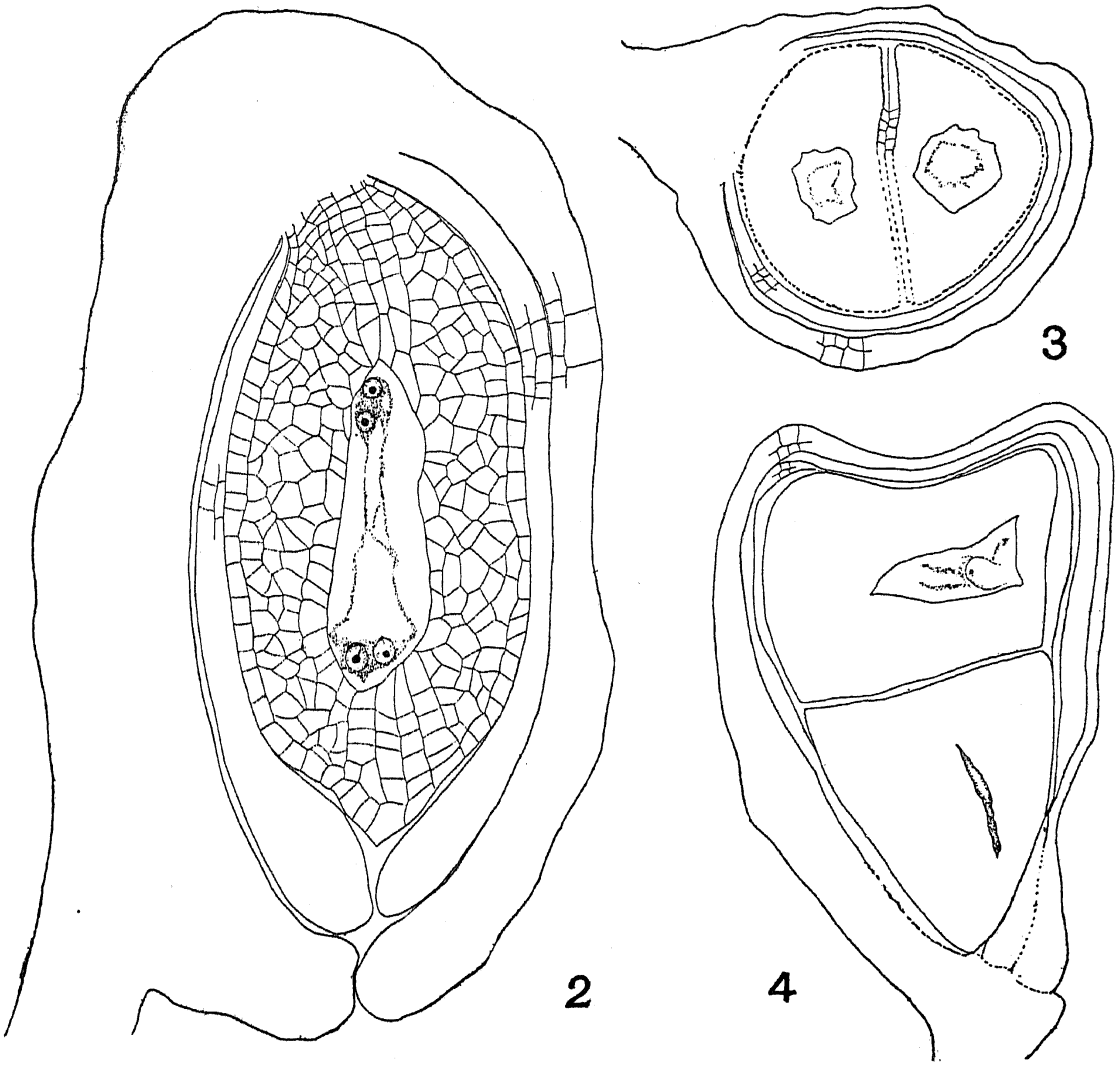
FIG. 1.—*Lagerstræmia Flos-Reginæ.*

Transverse section of the ovary, showing 7 loculi, 4 rows of anatropous ovules in each loculus, and the carpel midribs projecting inwards and forming partial septa. ×14.

There are two integuments and both take part in the formation of the micropyle (Figs. 1–6 and 31 *a* and *b*) just as in *Lawsonia*.[2] . At first both the integuments are two cells thick throughout their length. The inner integument remains so even in the mature ovule except near the micropyle, where some of the cells of its outer layer divide once, and it becomes three cells thick. Sometimes even the cells of its inner layer also divide, and for a very short distance it may become up to four cells thick. The cells of both the layers of the outer integument divide and through its greater length this integument becomes 4–5 cells thick, while at the micropyle it reaches the thickness of 7 to 8 layers of cells. The cells of this integument derived from the previous inner layer of cells are flattened parallel to the length of the ovule, while those derived from the outer layer are elongated anticlinally. In the latter close to the micropyle a deeply staining granular substance, probably of the nature of tannins, is deposited in *L. Flos-Reginæ*, as the ovule reaches maturity. No such deposits have, however, been seen in *L. indica* in the ovules showing mature embryo-sacs. This is the only point in which the integuments of the ovules of these two species differ. Otherwise they are quite similar.

The nucellus has got the same form as in *Lawsonia inermis*.[2] It is nearly straight except for a very slight curve towards the raphe at its chalazal end. The apex of the nucellus has always a characteristic pointed form (Figs. 2 and 31 *b*). On the sides of the embryo-sac, the nucellus consists of 5 layers of cells including the epidermis. Above the micropylar end of

the embryo-sac it is 5 or 6 cells thick in *L. indica* (Fig. 2) and about 7 to 8 cells thick in *L. Flos-Reginæ*. Its development in this region is described later. Below the embryo-sac, there are about 9–10 layers of nucellar cells in *L. indica* and about 10–12 layers in *L. Flos-Reginæ*. This part of the nucellus is characterised by the development of a strand of more or less regularly arranged and somewhat thick-walled cells (Figs. 2, 32 and 33), just as in *Lawsonia inermis*.[2] It also develops in the same manner as in



FIGS. 2–4.– *Lagerstræmia indica.*

FIG. 2.—Longitudinal section of an ovule containing a 4-nucleate embryo-sac showing its general form and structure of the nucellus. ×317.

FIG. 3.—Transverse section of an ovule in its upper part showing two nucelli lying side by side. ×145.

FIG. 4.—Longitudinal section of another similar ovule showing two nucelli, one lying above the other. ×145.

that species, and has probably got the same function of conducting the food supplies from the vascular bundle of the ovule ending in the chalaza to the base of the embryo-sac.

The union between the sides of the nucellus, the inner integument and the outer integument is not very fast. In the fixed and microtomed material they are generally found to separate from one another (Figs. 2–4).

Double Nucelli.

As in other plants, one nucellus within an ovule is the rule in species of *Lagerstræmia* also, but exceptions to this in *L. indica* are quite frequent and the writers have seen some ovules showing two nucelli within common outer and inner integuments. Two cases are sketched in Figs. 3 and 4. The first (Fig. 3) shows two nucelli placed side by side and cut transversely. Their epidermal layers are closely pressed against each other and for a large part are confluent and indistinguishable from the other nucellar cells. The embryo-sacs of both these nucelli were found to be perfect. In the second case (Fig. 4), the two nucelli are found to be placed one above the other and their epidermal layers are quite free from each other. The chalazal one has a normal embryo-sac, while the embryo-sac of the micropylar nucellus was found to be degenerating. The chalaza of the ovule containing these two nucelli was found to be very much lengthened towards the side of the raphe to accommodate the bases of the two nucelli.

Two nucelli within an ovule have also been seen in the family Lythraceæ by Mauritzon.[3] He found a pair of ovules in *Cuphea lanceolata* within the outer integument of which two nucelli were found surrounded by their own inner integuments, and another pair of ovules in *Cuphea petiolata* having two nucelli contained within common outer and inner integuments.

Megasporogenesis.

The primary archesporium differentiates simultaneously with the appearance of the initials of the integuments and the starting of the curving of the ovule (Figs. 7 and 22). It is multicellular and extends both to hypodermal and sub-hypodermal layers of cells, and thus agrees with the observations made by us on *Lawsonia inermis*.[2]

Usually only one archesporial cell develops further, while the rest gradually lose their archesporial characters and merge into the ordinary nucellar cells. Frequently, however, both in *Lagerstræmia indica* and *L. Flos-Reginæ*, as also happens in *Lawsonia*,[2] two archesporial cells in an ovule have been found to develop up to the megaspore-mother cell stage (Figs. 10 and 24) and rarely up to the embryo-sac stage (Fig. 21). The latter examples are described in greater detail further ahead.

The functional archesporial cell divides periclinally forming the primary wall cell towards the outside and the megaspore-mother cell towards the inside (Figs. 10 and 24). The primary wall cell divides first either anticlinally (Fig. 10, right hand side and Fig. 23) or periclinally (Fig. 8 and Fig. 24, right hand side). The first condition is more common. Next both periclinal and anticlinal divisions take place in rapid succession and give rise to a large amount of parietal tissue unlike what is seen in *Lawsonia*. It is 2–3 layers deep in *Lagerstrœmia indica* even before the megaspore-mother cell begins to divide (Fig. 9), is four layers thick when the megaspore-mother cell undergoes the heterotypic division (Fig. 11), five layers thick by the time the tetrad of megaspores is organised (Fig. 12), and remains in this condition up to the time of fertilisation (Fig. 2). In *Lagerstrœmia Flos-Reginæ*, the development of the parietal tissue is still more extensive and excluding the epidermis of the nucellus it reaches the thickness of 6–8 layers of cells (Fig. 31 *b*). There is no noteworthy degeneration of the parietal tissue before fertilisation due to the pressure of the growing embryo-sac as occurs in *Lawsonia*.

The megaspore-mother cell undergoes the two meiotic divisions in the normal manner (Fig. 11), and a linear tetrad of megaspores results (Figs. 12 and 25). Only one case of a T-shaped tetrad of megaspores has been noted in *Lagerstrœmia indica* (Fig. 13). As far as our observations go on these two species, there is no fluctuation in the selection of the functioning megaspore. The chalazal megaspore has always been found to be the functional one and the other three always degenerate (Figs. 12, 13 and 25). The degenerating megaspores persist up to the early 2-nucleate stages of the embryo-sac just as in *Lawsonia inermis*.[2]

In both the species of *Lagerstrœmia*, the megaspore-mother cell is generally supported by an axial row of regularly arranged cells (Figs. 9 and 23), which by further divisions give rise to the strand of conducting cells in the chalazal part of the nucellus described in the earlier part of the paper.

Development and Structure of the Embryo-Sac.

The functional megaspore develops vacuoles at both its ends (Fig. 12), and changes into the uni-nucleate embryo-sac. The bi-nucleate embryo-sac is often characterised by the persistence of the chalazal vacuole for an unusually long period (Fig. 26). The same feature has also been observed in certain other Lythraceæ by Mauritzon.[3] The 4-nucleate embryo-sac is normal and gives rise to the 8-nucleate embryo-sac.

The three antipodal cells are the first structures to differentiate in the embryo-sac, but they are quite ephemeral as in the other Lythraceæ and soon degenerate. In Fig. 17, they are seen to be degenerating when the

polar nuclei meet in the centre of the embryo-sac. They generally develop large vacuoles (Figs. 16 and 29). From the mature embryo-sac the antipodals are always absent (Fig. 18).

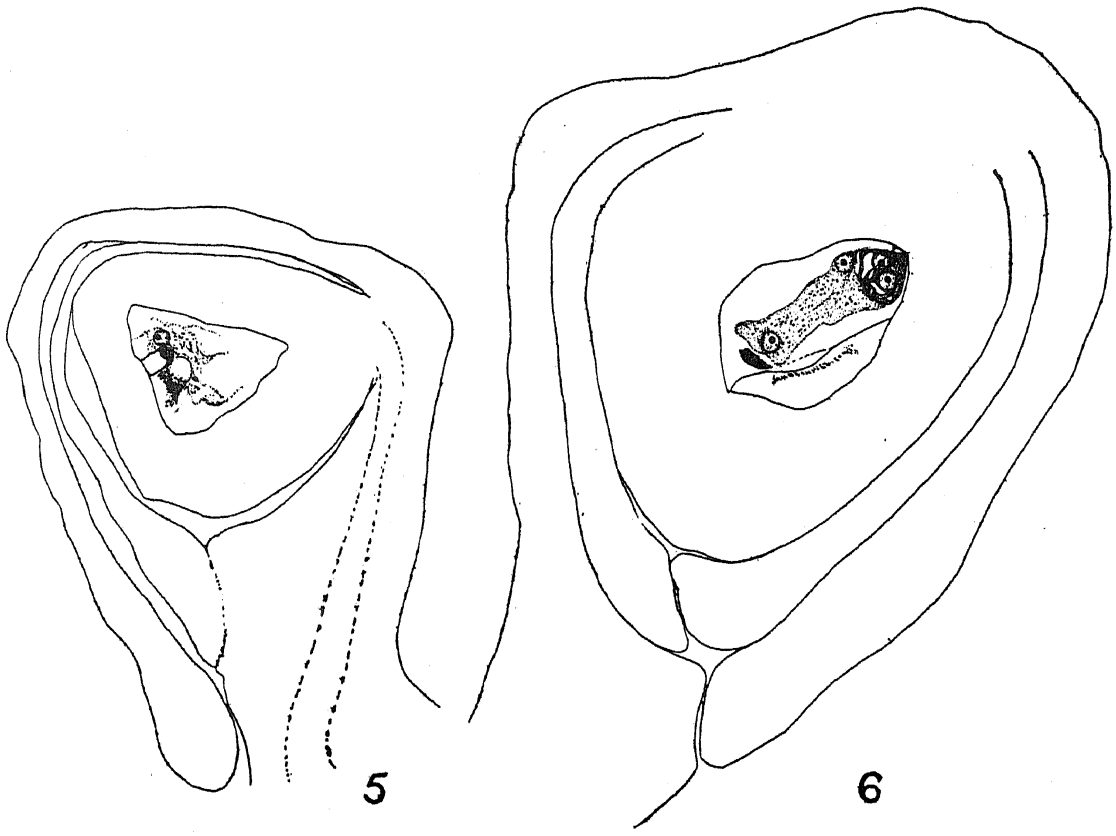
The polar nuclei meet about the middle of the embryo-sac (Figs. 17 and 29) and then move up and take a position below the egg-apparatus (Fig. 19). Here they remain free for a fairly long time and unite with each other to form the secondary nucleus only just before fertilisation (Figs. 20 and 30).

The egg-apparatus differentiates a little after the antipodals. The egg has the normal structure (Figs. 19, 20 and 30), and its mouth as in *Lawsonia inermis* is closed by a very thin membrane. The synergids are at first nearly pyriform (Fig. 16), but later on they become more elongated. The first part to differentiate in each of them is the chalazal vacuole (Fig. 16). Later on they develop hooks and the vacuoles in their pointed apices facing the micropyle of the ovule. A beginning of this micropylar vacuole formation is seen in Figs. 17 and 18 and a fully mature condition is shown in Figs. 19 and 30. The nuclei of the synergids as usual are situated in their micropylar halves.

The size of the embryo-sac at all stages in species of *Lagerstrœmia* is much larger than in *Lawsonia inermis*. While in the latter the embryo-sac at the time of fertilisation measures at the most 100 *u* in length, in *Lagerstrœmia indica* the mature embryo-sac is up to 175 *u* long and in *Lagerstrœmia Flos-Reginæ* 225 *u* long. The diameter of the embryo-sac varies from about $\frac{1}{5} - \frac{1}{4}$ of the length. The chalazal part of the embryo-sac in the later stages is generally broader than the micropylar part. In *L. Flos-Reginæ*, the mature embryo-sac at its chalazal end grows round the chalazal strand found in the nucellus of the ovule (Figs. 32 and 33), which consequently projects into the embryo-sac. This is due to the resistance offered by the conducting strand to the growth of the embryo-sac on account of the cells being somewhat thicker-walled than the other cells of the nucellus. In *L. indica* similar growth of the embryo-sac occurs at a little later stage, *i.e.*, during the development of the endosperm; and the same phenomenon has been observed by Mauritzon[3] in the genus *Cuphea*.

The mature embryo-sac does not show any notable amount of starch grains unlike what is seen in *Lawsonia*. The egg, synergids and the antipodals are surrounded by distinct cell-walls and the central cell containing the two polar nuclei separates from the rest in microtomed material due to the small amount of plasmolysis caused by the fixative.

Pollen-tubes in species of *Lagerstrœmia* are fairly broad and fertilisation is porogamous (Fig. 31 *b*).



FIGS. 5–6.—*Lagerstræmia indica.*

FIG. 5.—Longitudinal section of an ovule with rather an aberrant form, the micropyle is not situated exactly opposite to the chalaza and the egg-apparatus of the embryo-sac does not exactly lie below the micropyle. ×145.

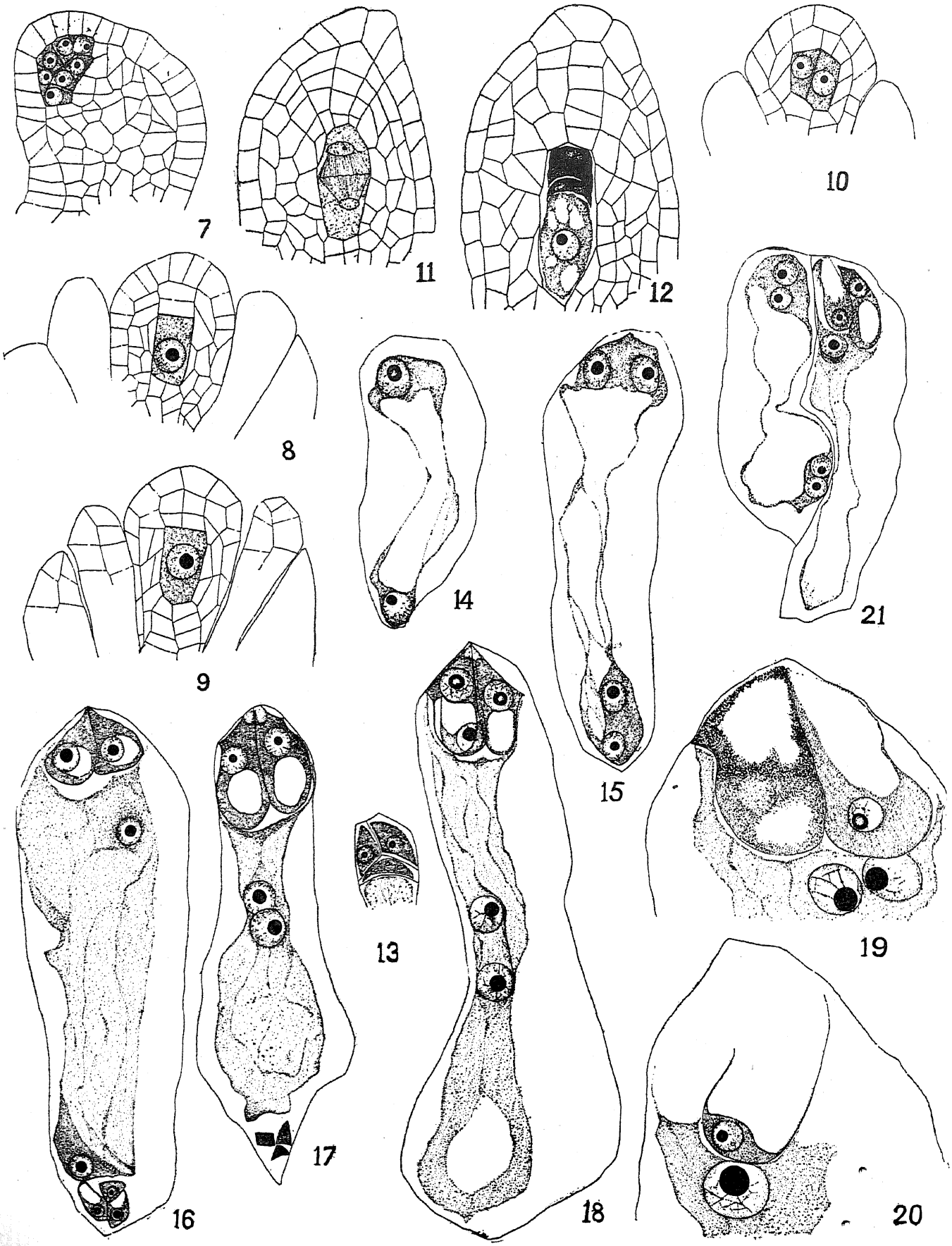
FIG. 6.—Longitudinal section of an ovule reconstructed from a number of adjacent sections showing two embryo-sacs, one of which shows reversed polarity. ×317.

Double Embryo-Sacs.

Double embryo-sacs are not so common in species of *Lagerstræmia* as in *Lawsonia inermis*,[2] but some instances have been seen by us in *Lagerstræmia indica*. Fig. 21 shows one mature and one 4-nucleate embryo-sac lying side by side. Both these embryo-sacs are quite healthy. Another instance of two embryo-sacs in the same ovule is seen in Fig. 6. The accessory embryo-sacs in all these cases had very probably developed from separate archesporial cells, and not from two megaspores of the same tetrad.

Exceptions to the Normal Polarity of the Embryo-Sac.

The polarity of the embryo-sac in the genus *Lagerstræmia*, as of course in the flowering plants in general, is quite fixed. The egg-apparatus is always situated at the micropylar end of the embryo-sac and the antipodals at the chalazal end. The polar nuclei are at first situated one at either pole,



FIGS. 7–21.—*Lagerstræmia indica.*

IG. 7.—Longitudinal section of a young ovule showing multi-cellular primary archesporium. × 633.

IGS. 8 & 9.—Micropylar parts of the longitudinal sections of two ovules showing megaspore-mother cell and parietal tissue at its different stages of development. × 633.

IG. 10.—Longitudinal section of an ovule showing two megaspore-mother cells lying side by side. × 633.

IG. 11.—Longitudinal section of the apical portion of the nucellus of an ovule showing the megaspore-mother cell in the heterotypic telophase and four layers of parietal tissue below the epidermis. × 633.

IG. 12.—Similar to the above, but showing a linear tetrad of megaspores, three of which are degenerating and the chalazal one is enlarging. × 633.

IG. 13.—A T-shaped tetrad of megaspores. The lowermost megaspore has not been cut properly. × 633.

IG. 14.—A 2-nucleate embryo-sac. × 633.

IG. 15.—A 4-nucleate embryo-sac. × 633.

IG. 16.—A young completely formed embryo-sac showing two synergids with a chalazal vacuole but still without a hook or the apical vacuole, two polar nuclei and three antipodals of which two show prominent vacuoles. × 633.

IG. 17.—A later stage of the same. The synergids are developing the hooks and the apical vacuoles. The polar nuclei are meeting in the middle and the antipodals are degenerating. × 633.

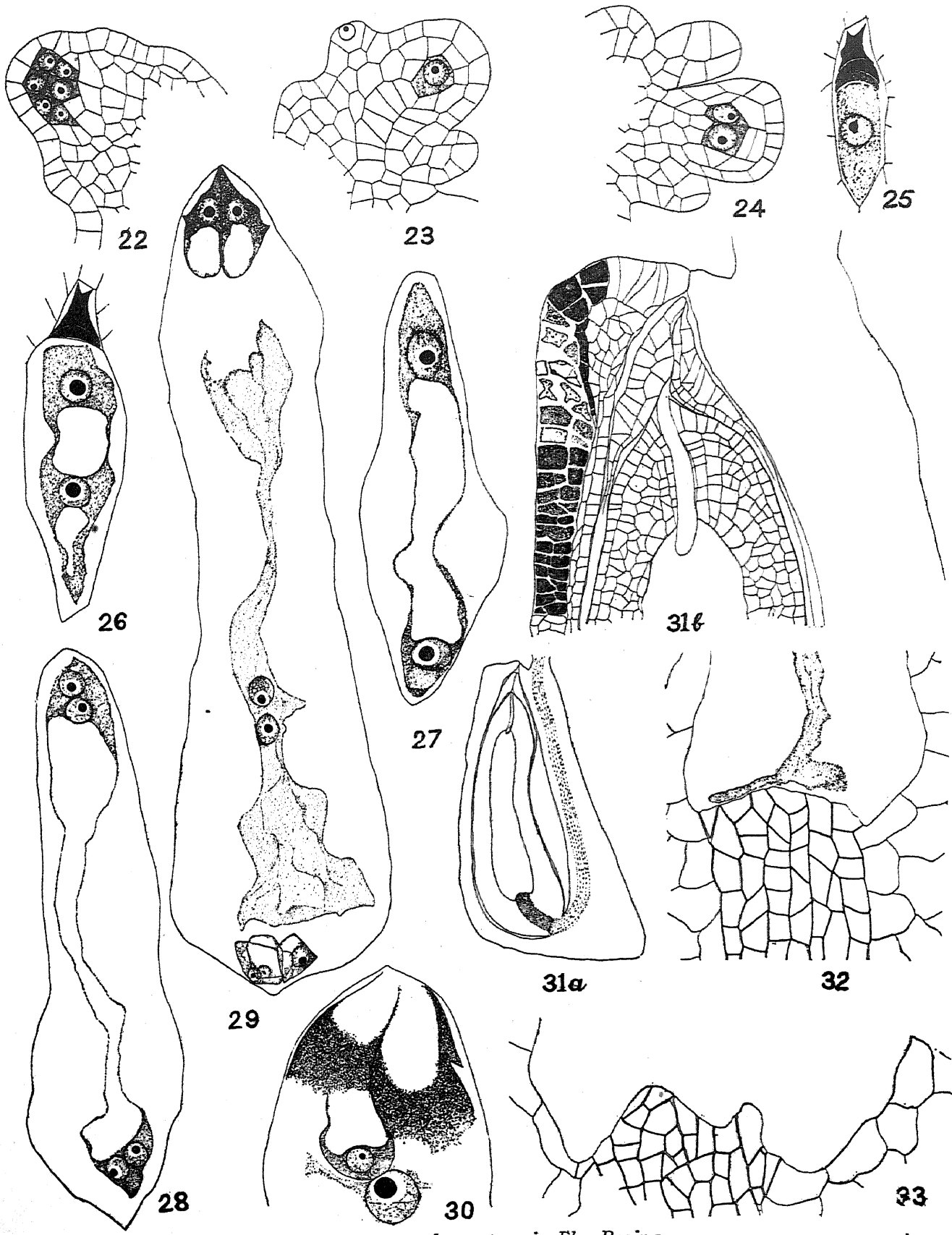
IG. 18.—Same as Fig. 17, but the antipodals have disappeared. × 633.

IG. 19.—Micropylar part of a nearly mature embryo-sac showing the egg, a synergid and two still free polar nuclei lying just below them. The synergid shows a prominent hook and a large vacuole in its micropylar apex. × 633.

G. 20.—Micropylar part of a still more advanced embryo-sac. The polar nuclei have fused. The synergids begin to degenerate about this time. × 633.

G. 21.—Two embryo-sacs from the same ovule lying side by side. One is fully formed; the other is 4-nucleate. × 633.

ıt ultimately, as described in the earlier part of the paper, they lie below ıe egg-apparatus. During the present study two exceptions have been noted / us to this general behaviour. In one case (Fig. 5), the ovule also had a mewhat aberrant form. It was not exactly anatropous and the chalaza was ɔt situated exactly opposite to the micropyle of the ovule. It contained somewhat triangular embryo-sac in which one of the angles pointed towards .e chalaza. The egg-apparatus was not exactly below the micropyle, but ced one side of the ovule opposite to the raphe. It had thus shifted its ɔsition towards one side. The secondary nucleus was seen close to the g-apparatus, while the antipodals had already degenerated. In the second se (Fig. 6) in an ovule with two embryo-sacs, one of the embryo-sacs ıs found to show completely reversed polarity. The embryo-sac was found be in an early stage of development. One polar nucleus was seen at :her end, but the antipodals were seen at the micropylar end and the egg-ɔparatus at the chalazal end.



FIGS. 22–33.—*Lagerstrœmia Flos-Reginæ.*

FIG. 22.—Longitudinal section of a young ovule showing a multi-cellular primary archesporium, initials of the inner integument and the beginning of the curving of the ovule. × 633.

FIG. 23.—Longitudinal section of a young ovule showing a megaspore-mother cell and two parietal cells. × 633.

FIG. 24.—Same as Fig. 23, but showing two megaspore-mother cells. × 633.

FIG. 25.—A linear tetrad of megaspores, with the upper three degenerating and the lowermost enlarging. × 633.

FIG. 26.—A young 2-nucleate embryo-sac showing three degenerating megaspores at its micropylar end, and an unusual persistence of the chalazal vacuole. × 633.

FIG. 27.—An advanced 2-nucleate embryo-sac. × 633.

FIG. 28.—A 4-nucleate embryo-sac. × 633.

FIG. 29.—A fully-formed embryo-sac showing two synergids with a large chalazal vacuole and a small hook but still without any apical vacuole, two polar nuclei meeting a little below the middle, and three antipodals with large vacuoles. × 633.

FIG. 30.—Micropylar portion of a mature embryo-sac, showing the egg, fused polar nuclei and two synergids with prominent hooks and apical vacuoles beginning to degenerate. × 633.

FIG. 31*a*.—A diagrammatic sketch of a longitudinal section of an ovule at the time of fertilisation cut along the raphe and showing its general structure. The shaded portion below the embryo-sac represents the strand of specially thickened and regularly arranged cells. The dotted portion in the raphe indicates the vascular bundle. A pollen-tube is seen in the micropylar part of the nucellus. × 53.

FIG. 31*b*.—Micropylar part of the ovule shown in Fig. 31*a*, showing the structure of the integuments and the nucellus. × 250.

FIG. 32.—A part of the longitudinal section of an ovule showing the chalazal end of the embryo-sac and the strand of specialised cells in the nucellus in this region. × 550.

FIG. 33.—Same as Fig. 32, but at a little more advanced stage. The chalazal end of the embryo-sac grows around the strand of specialised cells. × 550.

A similar case of reversed polarity in the embryo-sac in the family Lythraceæ has already been described by us in *Woodfordia floribunda*,[1] and other known cases are also mentioned in the same paper.

Degenerations in the Embryo-Sac.

Degenerations, which probably ultimately lead to the degeneration of the whole ovule, have been frequently met with in *Lagerstræmia indica*. In *L. Flos-Reginæ* these are comparatively rare. This may account for the abundant fruit formation in this species, while in the former fruit development is very much less. These degenerations in the embryo-sac occur at all stages of development, and their morphology is quite similar to what has been described in *Lawsonia inermis*.[2]

Summary.

The general structure and form of the ovule is quite similar to that of *Lawsonia inermis*,[2] but the parietal tissue is more extensive, 4-5-layered in

Lagerstræmia indica and 6-7-layered in *Lagerstræmia Flos-Reginæ*, and the outer integument becomes in the mature ovule 4–8 cells thick while the inner integument may also be 3–4 cells thick near the micropyle. A deeply-staining granular substance is deposited in the cells of the outer integument close to the micropyle in ovules of *L. Flos-Reginæ*.

The primary archesporium is many-celled. One cell only usually develops further, but frequently two cells develop up to the megaspore-mother cell stage, and rarely even into embryo-sacs, forming double embryo-sacs.

Megaspore-tetrads are linear, but one instance of a T-shaped tetrad has been seen in *Lagerstræmia indica*.

The chalazal megaspore always develops into the embryo-sac. The 2-nucleate embryo-sac is often characterised by the persistence of the chalazal vacuole for an unusually long period. The completely formed embryo-sac is of the normal 8-nucleate type. The antipodals possess large vacuoles and degenerate very early. They are always absent from the mature embryo-sac. The structure of the egg-apparatus is similar to that of *Lawsonia*. The polar nuclei at first meet about the middle of the embryo-sac. Later they travel upwards and take their position below the egg-apparatus, where they fuse just before fertilisation.

Fertilisation is porogamous.

Two cases of exceptional polarity of the embryo-sac have been recorded. In one of these the polarity of the embryo-sac was completely reversed.

Degenerations in the embryo-sac are more common in *Lagerstræmia indica* than in *L. Flos-Reginæ*.

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3. Mauritzon, J., "Zur Embryologie einiger Lythraceen," *Meddelanden Göteborgs Botaniska Trädgård*, 1934, **9**, 1-21.