EMBRYOLOGICAL STUDIES IN THE LYTHRACEÆ.

I. *Lawsonia inermis* Linn.

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In the family Lythraceae, the development of the embryo-sac in *Lythrum Salicaria* was investigated in 1917 by Tischler. He also made simultaneously a few unconnected observations on the embryo-sac of *Cuphea platycentra* and *C. cyanea*, and showed that in all these forms the antipodals degenerate very early and the mature embryo-sac, after the polar nuclei have fused, is only 4-nucleate. Besides this early degeneration of the antipodals, Tischler also observed certain other conditions in the embryo-sac of the species investigated by him which led him to emphasize that the embryo-sac of the Lythraceae forms a connecting link between the normal 8- (7-) nucleate embryo-sac and the 4-nucleate embryo-sac characteristic of the Onagraceae.

Until recently Tischler's investigation was the only important work on the embryology of the Lythraceae, but lately Mauritzon has published an account of the embryology of *Ammannia senegalensis*, *A. latifolia*, *Cuphea lanceolata*, *C. petiolata*, *C. platycentra*, *C. procumbens*, *Nesaea syphilitica* and *Peplis portula*, dealing in a comparative manner with megasporo-genesis, development and structure of the embryo-sac, endosperm, form of the nucellus and development of the embryo in these plants. Besides Tischler's and Mauritzon's researches, the development of the embryo-sac in the Lythraceae has been studied in *Cuphea Zinnapani* and *C. jorullensis* by Jönsson and Guignard respectively, but the work of these authors on account of its antiquity is very incomplete, and as Mauritzon has pointed out, it is also erroneous in certain parts. Souéges has studied the development of the embryo in *Lythrum Salicaria*, and his is the most comprehensive account about this part of the life-history in the Lythraceae.

The present studies on the embryology of the Lythraceae by the writers were started in 1933 much before the publication of Mauritzon's recent paper. They were undertaken on account of little embryological work on the family.
Lythraceæ as a whole, no work at all on the Indian representatives of the family, and the possibility of finding embryological peculiarities which are seen in the Onagraceæ. We have investigated the genera Lawsonia, Lagerstroemia, Ammania, Woodfordia, etc., and hope to publish their account one by one in the following papers. The last paper will include a discussion summarizing the results of all observations on the embryology of the Lythraceæ and a comparison with the embryological features of the allied families.

Lawsonia inermis Linn. (=L. alba Lamk.), the henna, which forms the subject of the present paper, is a glabrous shrub, probably a native of Persia, but is now widely cultivated in the plains of India, either as a hedge plant or for the sake of the dye that is found in its leaves and the strong scent that is emitted by its flowers. At Benares it flowers almost throughout the year producing large terminal pyramidal panicles of white, creamy or rosy flowers, about 3 inch across, with a broadly campanulate, 4-lobed calyx, 4 shortly clawed petals, 8 stamens inserted in pairs on the calyx-tube, opposite the calyx-lobes, and a 2-4-celled ovary containing many axile ovules.

The material used in this investigation was collected during 1933 from plants growing in the Benares Hindu University grounds and was fixed in Allen's modification of Bouin's fluid. The other processes of imbedding in paraffin and microtoming were carried out in the customary manner. Heidenhain's iron-alum-hæmatoxylin and a combination of safranin and gentian violet were mostly used for staining.

Development and Structure of the Normal Embryo-sac.

The organogeny of the flower is normal, the order of differentiation of the various floral parts being the calyx, the petals, the stamens and the gynoecium. The style is reflexed in the bud, but afterwards gets straightened.

The primary archesporium in the ovules differentiates just as the integument initials are formed (Fig. 4). Simultaneously with its differentiation, the ovule begins to curve. The archesporial tissue is many-celled and comprises both hypodermal and sub-hypodermal cells. Sometimes the whole of the nucellus excluding the epidermis at this stage seems to have the character of the archesporial tissue, all the cells possessing larger nuclei and taking a deeper stain than one usually finds in the non-archesporial cells. Such a condition is seen in Fig. 5. With further growth of the ovule all the cells composing the archesporium do not grow equally in size and pursue their development together. Generally one hypodermal archesporial cell becomes more prominent than the rest, and the others gradually merge into the ordinary nucellar cells. Exceptions to this behaviour, however, are very
frequent and will be described afterwards. The functional archesporial cell cuts off a primary wall cell and develops into the megaspore-mother cell (Fig. 21).

The primary wall cell divides at first anticlinally (Fig. 6), but afterwards its daughter cells undergo one periclinal division and thus give rise to a two-cell thick parietal tissue below the epidermis (Fig. 1). These cells become considerably elongated and are thus distinguished from the other cells of the nucellus situated on the sides of the embryo-sac. With the growth of the embryo-sac the inner layer of parietal cells is always crushed (Figs. 2, 10 and 11), and the embryo-sac at maturity is covered only by two layers of cells, the epidermis and the outer layer of parietal tissue (Fig. 2).

The cells surrounding the megaspore-mother cell are slightly richer in cytoplasm and stain somewhat deeper than the other cells of the nucellus and thus form a faintly differentiated tapetum (Fig. 6). This is crushed afterwards by the growth of the embryo-sac. Below the megaspore-mother cell differentiates a row of 2 or 3 cells which are much richer in cytoplasm than the other nucellar cells. The cells of this axial row undergo repeated divisions.
and give rise to a strand of slightly thick-walled, elongated cells rich in cytoplasm (Figs. 1, 2 and 3). This has most probably a conducting function, connecting the vascular supply of the ovule ending in the chalaza with the antipodal end of the embryo-sac.

The megaspore-mother cell undergoes the two meiotic divisions in the normal manner (Fig. 7) and gives rise to a linear (Figs. 8, 10 and 11) or less frequently a T-shaped (Fig. 9) tetrad of megaspores. During the heterotypic division the extrusion of karyotin bodies is frequently observed. The chalazal megaspore generally develops further into the embryo-sac and the other three on the micropylar side degenerate (Figs. 8, 9 and 10). Sometimes, however, the third megaspore from the micropylar end has been seen to enlarge (Fig. 10), and in a few cases both the third and the fourth megaspores from the micropylar end have been seen to enlarge simultaneously (Fig. 22). The cases of the latter type will be further considered under double embryo-sacs. The degenerating megaspores sometimes persist up to the early stages of the 2-nuc1eate embryo-sac (Fig. 11), but disappear during its further growth (Fig. 12).

The functional megaspore develops almost simultaneously two vacuoles, one at each end, and changes into the uni-nucleate embryo-sac (Figs. 8 and 9). Its nucleus undergoes a mitotic division. The two daughter nuclei move apart and a central vacuole appears (Fig. 11). The polar vacuoles are gradually crushed (Fig. 11) and the typical bi-nucleate embryo-sac results (Fig. 12). From this stage the development to the 8-nucleate stage is normal except for the degeneration of the antipodal nuclei at some stage or the other. We have seen very few instances of young complete embryo-sacs in which three antipodal cells are formed (Fig. 15). Often even in the 4-nucleate embryo-sac, the two nuclei at the antipodal (chalazal) end are found to be smaller than the two nuclei at the micropylar end (Fig. 13). This relation becomes more pronounced at the 8-nucleate stage. Sometimes while the nuclei at the micropylar end have completed their divisions and formed a quite healthy group of four nuclei, those at the antipodal end have been seen to dwindle and remain undivided (Fig. 14). It is not certain if these nuclei divide at all in the end, but their appearance does not show this possibility. We have also not been able to definitely decide if in such embryo-sacs a normal egg-apparatus is organised and both their egg and the single polar nucleus are fertilised. There is nothing, however, in the appearance of such embryo-sacs which precludes such a possibility. We do not believe in the possibility of a small polar nucleus from the lower end travelling upwards to meet the large micropylar polar nucleus, as we have not seen such a condition; and if it ever exists, it could have been easily detected.
Figs. 4-14. Lawsonia inermis.
The antipodals whenever they are present are seen only in very young embryo-sacs in which the egg-apparatus is not fully differentiated and the polar nuclei are still at their respective ends of the embryo-sac (Fig. 15). They disorganise and disappear very soon and no trace of them is seen even in a slightly older embryo-sac, such as, for instance, is seen in Fig. 16.

The polar nuclei appear to be interesting on account of their movements within the embryo-sac. At first the micropylar polar nucleus remains at its place and the chalazal moves upwards, and both meet just below the egg-apparatus (Fig. 16). They remain here for a short time, but afterwards together move down to the middle of the embryo-sac (Fig. 17). They may often pass down even slightly below the middle of the embryo-sac. Here they fuse with each other and give rise to a large central (secondary) nucleus (Fig. 18).

The egg-cell has the normal structure and is distinguishable by the presence of a large vacuole towards the micropylar end and the nucleus together with a small amount of cytoplasm pressed against the chalazal wall (Fig. 16). The neck of the egg seems to be closed at its micropylar end by a very thin membrane, so that in certain sections it often appears to be quite open.

The synergids form a very prominent part of the egg-apparatus and are always slightly longer than the egg-cell. As usual they have a large vacuole in their chalazal end and the nucleus and the cytoplasm are situated in the micropylar half. From an early stage the synergids develop indentations, which become more and more prominent with age (Figs. 16, 18 and 19). The apices of the synergids are pointed. At first these are full of cytoplasm (Fig. 16), but gradually the cytoplasm disappears (Fig. 18) and the synergids develop small apical vacuoles, which are seen most clearly in the synergids about to degenerate (Fig. 19). We have not seen the organisation of a distinct filiform-apparatus in this species. The nucleus of the synergids is generally bigger than that of the egg-cell. On degeneration the central portion of the synergids containing cytoplasm and the nucleus disorganises first and begins to stain very deeply (Fig. 19).

When first formed the embryo-sac is about 75 μ long and 16–18 μ in diameter. Its micropylar and chalazal ends are nearly equally broad (Fig. 15). Afterwards the embryo-sac broadens at the chalazal end (Figs. 16-18), reaching a diameter of 40 μ and its length at maturity is about 100 μ. The egg-apparatus just before fertilisation is about 20 μ long.

The mature embryo-sac is full of starch grains. The cells of the egg-apparatus and the antipodals are all surrounded by distinct walls of cellulose. The central cell with the two polar nuclei, therefore, is always quite separate
from the rest and in the fixed material shrinks to some extent from the other structures at the two ends.

**Double and Triple Embryo-sacs.**

Although generally only one primary archesporial cell develops further into a megaspore-mother cell and the ovules show only a single embryo-sac, exceptions to this condition are quite common in *Lawsonia inermis*. Often two or three archesporial cells begin to develop further. These are usually all hypodermal and give rise to megaspore-mother cells and later on to tetrads of megaspores lying side by side. The first condition is seen in Fig. 21. This is a sketch of a longitudinal section of an ovule showing two megaspore-mother cells which have cut off the primary wall cells. In Fig. 23 two tetrads of megaspores are seen lying side by side. The upper three megaspores of each tetrad have degenerated while the chalazal one has developed into a uni-nucleate embryo-sac.

Sometimes a sub-hypodermal archesporial cell may also begin to develop along with a hypodermal archesporial cell and may give rise to a megaspore-mother cell and later on to an embryo-sac. This is seen in Fig. 20. Here two archesporial cells lying in a row are seen to be developing simultaneously. The case sketched in Fig. 24 has probably resulted from such a condition. The upper archesporial cell after cutting off the parietal cell has developed into a tetrad of megaspores, the upper three of which have degenerated and the lower has developed into a uni-nucleate embryo-sac; and the lower archesporial cell which has lagged behind in development has cut off perhaps a parietal cell and has developed into a megaspore-mother cell.

Twin embryo-sacs in *Lawsonia inermis* are also sometimes formed by the simultaneous development of two sister megaspores of the same tetrad. This is seen in Fig. 22. The upper two megaspores of a tetrad in this case have degenerated, while both the chalazal ones have developed into bi-nucleate embryo-sacs.

The best examples of multiple embryo-sacs that we have observed during the present investigation are sketched in Figs. 25 and 26. Figs. 25a and 26a show the form of the entire ovules showing such embryo-sacs at a small magnification. In Figs. 25b and 26b, the embryo-sacs from these ovules are shown at a higher magnification. Figs. 25a and b show the presence of two embryo-sacs within the same ovule. One of these, on the right in the figure, is in the eight-free-nucleate condition. The other on the left shows a poorly organised egg-apparatus and the antipodal cells. From the position of the embryo-sacs it appears that these have been derived from separate megaspore-mother cells. In Figs. 26a and b, three embryo-sacs
Figs. 15–25. *Lawsonia inermis*.
are seen within the same ovule. One of these on one side is 8-nucleate and is more or less in a degenerated condition. The other two are in a row, one above the other. The upper one is bi-nucleate, while the lower (chalazal) one is 4-nucleate. The 8-nucleate embryo-sac which is seen on the left has certainly come from a separate megasporocyte as compared with the other two, but it is not certain how the latter have developed. They may have come from separate megasporocytes lying in the same row such as are seen in Fig. 24, or from two sister megaspores of the same tetrad. On account of the upper embryo-sac being 2-nucleate and less advanced than the lower, the possibility for the second interpretation to be right seems more likely.

The ovules showing double and triple embryo-sacs are of the same size as the normal ones showing only a single embryo-sac. The fate of the accessory embryo-sacs cannot be definitely described. It probably follows no definite rule, but a common condition is that all the embryo-sacs of ovules showing more than one embryo-sac sooner or later degenerate. This is due to physiological reasons. The quantity of food coming into an ovule remains the same. It is possibly enough only for one embryo-sac, but as it is distributed to more than one, no embryo-sac gets its proper supply and all degenerate.

Degenerations in the Embryo-sacs.

Besides the degenerations which affect the antipodal part of the embryo-sac and which are probably of an orthogenetic nature, other degenerations
of a physiological nature are also quite commonly seen in the sporogenous cells and the embryo-sacs of *Lawsonia inermis*. These lead to the complete degeneration of the parts affected by them and are seen at all stages in the development of the embryo-sac, from the megaspore-mother cell stage onwards. A few of these are sketched here. Fig. 27 shows a tetrad in which all the megaspores are degenerating. Fig. 28 shows a degenerating un-nucleate embryo-sac, and Fig. 29, a degenerating bi-nucleate embryo-sac. In Fig. 30 is represented a degenerating 4-nucleate embryo-sac and Fig. 31 shows an almost completely degenerated mature embryo-sac.

During these degenerations the nucleus is affected first. Its outline begins to become irregular and indistinct. Along with the surrounding cytoplasm, its staining capacity increases. Finally in the completely degenerated parts, the nuclear outline disappears, and the whole nuclear and cytoplasmic mass stains intensely.

The Ovule and the Nucellus.

The mature ovules containing embryo-sacs ready for fertilisation are about 315 \( \mu \) long. They are anatropous. Their form is slightly variable owing to their being closely pressed against one another in the ovary, but Fig. 2 gives a general idea of their shape. They are nearly cylindrical and not markedly flattened in any plane, as are the ovules of *Cuphea* according to Mauritzon.³ They have a chalazal end, however, is markedly broader than the micropylar end, and in this respect they differ from the ovules of *Nesaea* and *Peplis* with which they closely resemble otherwise. They are arranged on the placentas more or less horizontally with the micropyle pointing downwards. Their both integuments are two cells thick and grow beyond the nucellus. The micropyle is thus formed by both the integuments (Figs. 1 and 2).

The nucellus is nearly straight except for a small curve towards the chalaza. On the sides of the embryo-sac it consists of 4 or 5 layers of cells including the epidermis. Above the embryo-sac, as described previously, during the early stages (Fig. 1) excluding the epidermis there are two layers of parietal tissue. At the time of the mature embryo-sac, the inner one of these is disorganised by the growth of the embryo-sac and there is only a single layer of parietal tissue above the embryo-sac. The cells of this layer are larger than those in the other parts of the nucellus, are conspicuously elongated and are seen to be arranged in a radiating manner. Below the embryo-sac there are about 10 layers of nucellar cells. This region is conspicuous by the presence of a strand of elongated cells, whose structure and development has already been described.
Summary.

The ovules of *Lawsonia inermis* are anatropous and both the integuments take part in the development of the micropyle. The mucellus is 4–5 cells thick on the sides of the embryo-sac and about 10 cells thick below the embryo-sac. The latter region is distinguished by the development of a strand of somewhat elongated, regularly arranged, deeply staining cells. Above the embryo-sac, besides the epidermis, there are two layers of parietal tissue in the early stages, but afterwards the inner one is crushed.

The primary archesporium is many-celled, consisting both of hypodermal and sub-hypodermal cells. Only one of these usually cuts off the wall cell and gives rise to a megaspore-mother cell. Exceptions to this behaviour, however, are quite common, and more than one megaspore-mother cells, tetrads of megaspores, and embryo-sacs are quite commonly seen in one ovule.

The megaspores are generally arranged in linear tetrads, but T-shaped tetrads also are sometimes seen.

The chalazal megaspore mostly develops into the embryo-sac and the others degenerate, but sometimes the second megaspore from the chalazal end and sometimes both the chalazal megaspores have been seen to function.

The development of the 8-nucleate embryo-sac is normal except for the frequent lagging behind and degeneration of the nuclei of the antipodal end. Often at the 4- and 8-free-nucleate stages the chalazal nuclei of the embryo-sac are smaller than the micropylar nuclei. Sometimes while passing from the 4-nucleate to the 8-nucleate stage, while the micropylar nuclei undergo the usual division, the chalazal ones degenerate. The antipodal cells whenever they are formed are very transitory structures and soon degenerate. The egg has got the normal structure. The synergids are hooked and develop, besides the large chalazal vacuole, a small vacuole in their pointed apices. The polar nuclei at first meet below the egg-apparatus, but afterwards pass down and fuse about the middle of the embryo-sac to form a secondary nucleus. Starch grains are deposited in the mature embryo-sac.

Double and triple embryo-sacs in an ovule, either due to the development of more than one archesporial cell, or due to the functioning of two megaspores of a tetrad, are quite frequent.

Degenerations in the embryo-sac are met with commonly and take place at all stages of development.
LITERATURE CITED.

EXPLANATION OF FIGURES.

**FIG. 1.**—Longitudinal section of an ovule showing its structure at the time of development of the uni-nucleate embryo-sac. The three degenerating megaspores are still present. Both the integuments are two cells thick and both take part in the formation of the micropyle. There are two layers of parietal tissue above the tetrad of megaspores. Below the chalazal megaspore (uni-nucleate embryo-sac) are seen 3-4 rows of elongated cells. The vascular bundle of the ovule is stippled in the figure. ×317.

**FIG. 2.**—Shows the longitudinal section of an older ovule containing a nearly mature embryo-sac. The latter shows two synergids and two polar nuclei in the process of fusion. The antipodals always disappear by this time. The egg was seen in an adjacent section. The inner layer of parietal cells has disappeared, while the conducting strand in the chalazal part of the nucellus is fully developed. In other respects it resembles Fig. 1. ×317.

**FIG. 3.**—Sketch of the portion of the nucellus just below the embryo-sac from a mature ovule showing the structure of the conducting strand in detail. The cells in this part are somewhat elongated, regularly arranged, possess denser cytoplasm and stain deeper than the other nucellar cells. ×633.

**FIG. 4.**—Apical part of a young ovule showing a multicellular hypodermal and sub-hypodermal primary archesporium and the initials of the two integuments. ×830.

**FIG. 5.**—Apical part of a slightly older ovule than the one sketched in Fig. 4, showing the whole nucellus excepting the epidermis having the character of the archesporial tissue. ×830.

**FIG. 6.**—A still older ovule showing the megaspore-mother cell in synizesis, two parietal cells formed from the primary wall cell, a tapetal layer surrounding the megaspore-mother cell and an axial row of cells below the megaspore-mother cell. The integuments have grown up to the sides of the nucellus. ×830.

**FIG. 7.**—A megasporocyte in the telophase of the second meiotic division. ×830.

**FIG. 8.**—A linear tetrad of megaspores with the upper three degenerating and the lower developed into the uni-nucleate embryo-sac. ×830.

**FIG. 9.**—A T-shaped tetrad of megaspores. Otherwise similar to Fig. 8. ×830.

**FIG. 10.**—A tetrad of megaspores with the second cell from the chalazal end developing into the embryo-sac. ×830.

**FIG. 11.**—A bi-nucleate embryo-sac with the three degenerating megaspores still present over its micropylar end. ×830.
Fig. 12.—An older stage of the bi-nucleate embryo-sac. × 830.

Fig. 13.—A 4-nucleate embryo-sac with the apical part of the nucellus. The two micropylar nuclei in the embryo-sac are bigger than the chalazal nuclei. × 830.

Fig. 14.—A later stage of the same. The micropylar nuclei of the embryo-sac have given rise to a group of four, while the two chalazal nuclei are dwindling and have not divided. × 830.

Fig. 15.—A young 8-nucleate embryo-sac showing early stages in the differentiation of the egg-apparatus, three antipodal cells, and two polar nuclei still at their respective poles of the embryo-sac. × 830.

Fig. 16.—A later stage of the embryo-sac. The antipodals have disappeared. The chalazal polar nucleus has moved up to the micropylar polar nucleus and both are seen just below the egg-apparatus. The egg is showing a large vacuole in its micropylar part, while the synergids is showing a pointed apex still full of cytoplasm, a horizontal indentation and a nucleus slightly above its middle, and a large vacuole in the chalazal end. × 830.

Fig. 17.—Lower part of a still older embryo-sac. The chalazal end has greatly broadened. The polar nuclei have moved to below the middle of the embryo-sac. Starch is abundantly present. × 830.

Fig. 18.—An embryo-sac slightly older than the one shown in Fig. 17. The polar nuclei have fused and have given rise to a secondary nucleus situated near the middle of the embryo-sac. The egg-apparatus shows two synergids with vacuoles developed in their apex. Starch grains are seen to be abundantly present as in the previous case. × 830.

Fig. 19.—Synergids at the time of degeneration. × 830.

Fig. 20.—Apical part of a young ovule showing two archesporial cells situated in a row enlarging. × 830.

Fig. 21.—Apical part of an ovule showing two young megaspore-mother cells lying side by side. The primary wall cells are seen above them. × 830.

Fig. 22.—A tetrad of megaspores with the two chalazal megaspores developed into 2-nucleate embryo-sacs. × 830.

Fig. 23.—Two tetrads of megaspores lying side by side. The upper three of each have degenerated, while the chalazal ones are developed into uni-nucleate embryo-sacs × 830.

Fig. 24.—A probable row of two archesporial cells. The upper one, after cutting off the primary wall cell, has given rise to a row of four megaspores, the upper three of which have degenerated, while the lower one has developed into a uni-nucleate embryo-sac. The lower archesporial cell has cut off a primary wall cell and has developed up to the megaspore-mother cell stage. × 830.

Fig. 25a.—An ovule showing two embryo-sacs. × 85.

Fig. 25b.—The embryo-sacs of the above shown at a higher magnification. One on the right is in the 8-free-nucleate stage. The other shows the differentiation of the egg-apparatus, etc. × 830.

Fig. 26a.—An ovule showing three embryo-sacs. × 85.

Fig. 26b.—The embryo-sacs of Fig. 26a shown at a higher magnification. One is situated on one side and is 8-nucleate. The other two are in a row. The upper is 2-nucleate, while the lower is 4-nucleate. × 830.

Fig. 27.—A tetrad showing all the megaspores degenerating; FIGS. 28, 29, 30 and 31. 1-nucleate, 2-nucleate, 4-nucleate and mature embryo-sacs respectively in various stages of degeneration. × 950.