STRUCTURE AND DEVELOPMENT OF THE SYNERGIDS IN *AMMANIA BACCIFERA* LINN.

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(With Plate III, containing Figs. 1–10)

The following communication relating to the structure and development of the synergids in the embryo-sac of *Ammania baccifera* Linn., a marsh annual herb belonging to the family Lythraceae, is being written on account of the exceptional behaviour of these parts of the embryo-sac in this plant. This fact was recorded by us some time ago in a brief note elsewhere (4). Details and figures are given in the present paper.

The ovules of *A. baccifera* are of the normal anatropous form with two integuments and a moderate amount of nucellus. They show many primary archesporial cells, one of which usually develops to form the primary parietal cell and the megaspore mother cell. The latter gives rise to four or three megaspores. Out of these, the chalazal megaspore is always the functional one and develops into the 8-nucleate embryo-sac in the normal manner, forming three antipodals, an egg apparatus of an egg cell and two synergids and two polar nuclei. A tapetum is differentiated even at the megaspore mother cell stage and persists up to the complete formation of the embryo-sac. The embryo-sac is at first narrow at both the ends but later on broadens at the micropylar end. The antipodals are at first arranged in a row, but in the mature embryo-sac they form a triangle. The polar nuclei take their place near the egg apparatus. The egg cell has the normal structure and shows a large vacuole towards the micropylar end and the nucleus at the lower end.

The synergids, on the other hand, differ markedly from those of other flowering plants investigated so far. At an early stage in the development of the embryo-sac, soon after the 8-free-nucleate stage and much before the egg cell is ready for fertilisation, the two synergids are quite free from each other. They lie side by side in their usual position and in frontal view completely cover the egg cell, except at its lower end (Fig. 1). The size of the egg cell and the synergids is about the same, both being about 6 μ in length. Laterally the syner-

gids touch each other on one side, but are separate on the opposite side. A peculiarity of the synergids visible even at this stage and one that persists throughout their later life is the absence of vacuoles. As a rule, there is no trace of them. Only in one instance, a small vacuole in one of the synergids has been seen (Fig. 3). Correlated with the above fact, the nuclei of the synergids are not situated close to their micropylar ends. They are found about the middle (Fig. 2) or even somewhat near the chalazal end. The whole of the plasma of the synergids is uniformly very dense and stains deeply.

The free condition of the synergids lasts for a very short time. After examining hundreds of ovules, we have seen it only in a few instances. Very soon they begin to grow in size and to press upon each other laterally. Ultimately the line separating them vanishes (Fig. 2) and they fuse with each other (Figs. 2 and 3). The fused structure, which may be now called a "syn-synergid", enlarges very much in size, keeping pace with the growth of the egg cell and the embryo. At certain stages, it is even larger than the egg cell (Fig. 3). The free synergids (Fig. 1) are only about 6 μ long. By the time the fertilisation takes place (Fig. 4), the syn-synergid is about 25 μ in length and diameter. Fully formed synergids measure from 50 to 60 μ in length and breadth (Figs. 6 and 7).

The syn-synergid covers the egg and later on the proembryo and the embryo on one side (front side) completely (Fig. 6) and on the opposite side partially. Around the suspensor of an embryo, the syn-synergid is equally broad all round, but lower down about the base of the embryonal mass it is much thicker on one side than on the other. This can be seen from Figs. 8 and 9, which represent cross-sections of a syn-synergid, one at the level of the suspensor (Fig. 8) and the other at the level of the embryonal mass (Fig. 9). A complete diagram of the syn-synergid is given in Fig. 5. This is a reconstruction from two adjacent sections and shows the synergids at the three-celled stage of the proembryo in the sagittal plane. The whole thing looks like a collar with one side broader than the other, surrounding the base of the embryo, with a narrow opening at the top into which the micropylar end of the embryo fits and a broad opening on the opposite side through which the embryonal cell (and, later on, the embryonal mass) projects.

The syn-synergid is binucleate when it is just formed, one nucleus coming from each synergid. These nuclei are, at first, in their original position, about the middle of each synergid, but now they move downwards towards their base (Fig. 3). They show a gradual increase

in size, and ultimately begin to divide and multiply in number as the syn-synergid rapidly enlarges. The syn-synergid thus becomes a multinucleate coenocyte, the number of nuclei reaching about 20. From 15 to 17 nuclei in a syn-synergid just as the periblem and plerome are forming in the embryo (Fig. 6) are quite common. The divisions that give rise to such a large number of nuclei from the original two, take place amitotically by a process of budding (Figs. 5, 6 and 9). During this process, the nuclear cavity of the mother nucleus becomes perfectly hyaline, probably due to the flow of the whole of the chromatic material into the nucleolus. This nucleolus buds off one or more round daughter nucleoli resulting in the presence of two or more daughter nucleoli within the cavity of the mother nucleus. Constrictions in the wall of the mother nucleolus now appear around the individual daughter nucleoli. These gradually increase in size and lead ultimately to the separation of the daughter nuclei from the mother nucleus. These daughter nuclei now move apart and repeat the process. As the syn-synergid reaches its maximum size, multiplication in the number of the nuclei stops. These now begin to grow in size, reaching a diameter of 8μ and develop a faint chromatin reticulum (Fig. 7).

The nuclei in the syn-synergid are distributed more abundantly on one side than on the other (Figs. 5, 6, 8 and 9). This is the frontal side on which the synergids previously in the free condition were situated and on which side the syn-synergid is thicker (Fig. 9). This unequal distribution of the nuclei in the syn-synergid is quite natural, as the two parent nuclei were on this side and all the daughter nuclei have radiated out from them.

Finally, the syn-synergid degenerates when the embryo has reached the stage represented in Fig. 10. Dermatogen, periblem and plerome have become fully established at this stage in the embryonal mass. The hypophysis cell has divided into two and one of its daughter cells has already divided longitudinally.

The above behaviour of the synergids in the embryo-sac of *Ammania baccifera* has been found to be quite constant, and no exception has been observed so far. The process starts, as said previously, at a very early stage, much before the beginning of the process of fertilisation and not after the completion of this process, as was stated by us previously (4). This can be seen from Fig. 4, which represents a stage in the process of fertilisation. The section is from one side of the egg apparatus and the egg cell and the synergids have not been cut in the median plane. The apex of the stout pollen tube

is still intact and it has not as yet discharged its contents. The synergids have already fused, measure about 25 μ in length and diameter, and on counting 9 nuclei have been seen in this particular case.

In preparations stained with Haidenhain's iron alum haematoxylin, during early stages, the syn-synergid and the embryo take nearly the same amount of stain. The rest of the embryo-sac stains very lightly. During later stages, as the time of their degeneration draws near, the staining capacity of the synergids decreases. A similar reaction is given by iodine solution. This stains the synergids and the embryo yellowish brown, indicating only the presence of dense plasma. Starch grains are totally absent from these two parts, though they are present in other parts of the ovule, particularly in the neighbourhood of the vascular elements in the funicle.

Comparison and discussion

In general, the synergids in the embryo-sac of angiosperms, with regard to the method of their formation, structure and position, show very little variation. Their position at the apex of the embryo-sac, their vacuoles situated towards their chalazal ends and the position of their nuclei in the micropylar region, are peculiarities which have been found to reappear with very few exceptions in the numerous flowering plants which have been investigated by now. In most instances further, the synergids have been found to degenerate as soon as the process of fertilisation is complete. For these reasons any variation in the structure of the synergids is worth recording.

The chief features of the synergidae of *Ammania baccifera* are the absence of vacuoles, their great increase in size, their fusion with each other to form a single structure, the multiplication of their nuclei by a process of amitosis and their persistence up to a fairly late stage of embryo formation. It may be immediately stated, that so far as the writers are aware, these features all together have not been recorded in any other flowering plant. In the family Lythraceae, the embryo-sac of *Lythrum Salicaria* has been studied in detail by Tischler (14) and he has also made some comparative observations on species of *Cuphea*. The embryo-sac of some more species of *Cuphea* has been studied by Jönsson (3) and Guignard (1). We have ourselves by now examined the embryo-sac of *Lawsonia alba*, *Woodfordia floribunda*, and *Lagerstroemia Flos-reginae*. In all these plants normal synergids have been found to occur. The morphology and cytology of several

other families of the Myrtales is well known, and although several other anomalies have been observed in their embryo-sacs—e.g. the embryo-sac in the Onagraceae is only 4-nucleate (2, 6, 8), in the Penaeaceae it is 16-nucleate (12), etc.—but in no case synergids of the type, such as have been found in *Ammania baccifera*, have been reported. A comparison in some respects, however, can be made with certain unrelated plants.

The absence of vacuoles in the synergids is a very rare feature, but synergids which completely lack the vacuoles are present in *Limnanthes Douglasii*, according to Stenar (11). The lower part of the synergids in this plant is spherical and swollen, and is filled completely with dense plasma. Also in this plant the nuclei show hypertrophic development. In *Lycopsis arvensis*, the conditions, according to Svenson (13), are somewhat similar. The synergids are more strongly developed than the egg cell and possess very small and not sharply defined vacuoles of varying position, and their nuclei are early hypertrophied.

The enlargement and persistence of the synergids are already known in *Trapella*. According to Oliver (5), the synergids in this plant after fertilisation increase much in size and in the mature seed form a conspicuous tubercle-like body. Schürhoff (9) has similarly observed the persistence, strong growth and intense stainability of the synergids and a great increase in the size of their nuclei, often becoming bigger than the complete synergid in the beginning, in *Allium odorum*.

The fusion of the two synergids at a very early stage and a large multiplication in the number of their nuclei, however, has been observed in no other plant, and in these respects the synergids of *Ammania baccifera* appear to be unique.

The function of this peculiar development of synergids in *Ammania baccifera* seems to be obscure, and it is rather difficult to put forward any definite view. In the various examples in which the synergids have been found to persist, enlarge in size, have deeply staining plasma, no vacuoles and nuclei showing hypertrophy, it has been generally believed that they help in the nutrition of the egg, Schnarf (7). In *Allium odorum*, for instance, the endosperm develops very late, after the embryo has already undergone a number of cell divisions and Schürhoff (9) assumes that these synergids take over the function of endosperm. In *Ammania baccifera* the endosperm is very scantily developed, and during early stages of embryogeny, while the rest of the embryo-sac is very poor in plasma, the synergids

stain as deeply as the egg cell or the embryo, both with haematoxylin and iodine solution. It is, therefore, quite probable that the synergids in this plant also may be playing some role in the nutrition of the egg.

As regards the occurrence of amitosis, it is usually associated with tissues having a nutritive function or with pathological and degenerating tissues. It has, therefore, been put forward by some workers that amitosis aids in the process of metabolism by increasing the nuclear surface and by others that it is primarily a degenerative phenomenon, Sharp(10). The synergids of *Ammania baccifera*, as stated above, may be for some time performing a nutritive function. Ultimately they degenerate. The amitotic multiplication of nuclei in them may be, therefore, correlated with both of the above explanations and its occurrence may be regarded as quite expected.

Summary

The embryo-sac of *Ammania baccifera* develops from the chalazal megaspore in the normal manner. It is of the 8-nucleate type. All its parts have the usual structure except the synergids. Their free condition, which is normal in other plants, lasts only for a short time. Very soon they fuse with each other laterally and form a sort of collar around the egg and later on around the base of the growing embryo. They show great increase in size from about 6 to 60 μ. They are peculiar in the absence of vacuoles and presence of a uniform dense plasma, in the multiplication of their nuclei by amitotic divisions and in persisting up to a fairly late stage of embryo development. Such a behaviour of synergids has not so far been recorded in any other flowering plant.

Note on the figures. All the figures are camera lucida drawings, but Figs. 5 and 6 are reconstructions from a number of adjacent sections to show the whole form of the syn-synergid and the total number of nuclei. The embryo figures are not shaded. Magnification of all the figures is about 1150 times.

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EXPLANATION OF PLATE III

Fig. 1. *Ammania baccifera*. Micropylar part of the embryo-sac, showing the egg apparatus and two polar nuclei. The synergids as yet are quite free from each other.

Fig. 2. A slightly later stage than that shown in Fig. 1; the line separating the two synergids has dissolved and they have fused.

Fig. 3. The egg apparatus at a stage later than the one represented in Fig. 2, cut from one side, not medianly. The synergids are now much larger and fused with each other. One of them shows the exceptional presence of a vacuole. There are two nuclei towards the base.

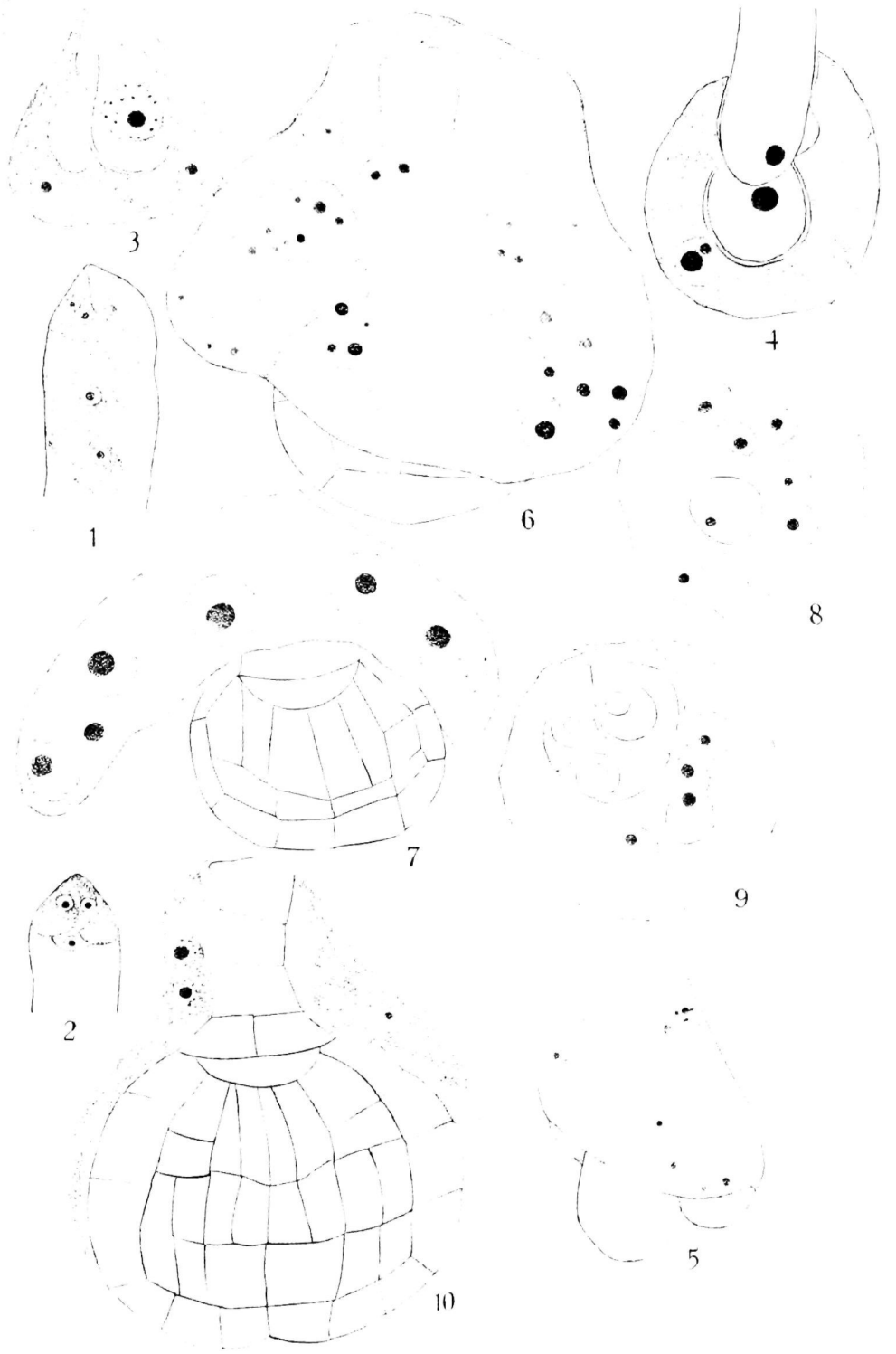
Fig. 4. A stage in the process of fertilisation as seen from one side. The synergids at this stage show about 9 nuclei.

Fig. 5. The syn-synergid at the 3-celled stage of the proembryo in sagittal view.

Figs. 6 and 7. Later stages in the development of the syn-synergid.

Figs. 8 and 9. Cross-sections of one syn-synergid. Fig. 8. At the level of the suspensor. Fig. 9. About the base of the embryonal mass. Fig. 9 shows that at this level the syn-synergid is much thicker on one side than on the other. Both the figures show that nuclei are more abundant on one side (thicker side) than on the other.

Fig. 10. Syn-synergid at the time of degeneration.



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