

## STUDIES IN THE FAMILY AMARANTHACEÆ.

### I. The Life-History of *Digera arvensis* Forsk.

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#### 1. *Introduction.*

Of all the families belonging to the order Centrospermales, Amaranthaceæ is the one, which has so far attracted the least attention from morphologists. In 1859 Braun\* observed polyembryony in *Celosia*. In 1880 Fischer\* found an axial row of only three megasporangia in *Gomphrena decumbens*. In 1882 Guignard\* studied the development of the embryo sac and endosperm in *Celosia argentea*. Dahlgren (1916) reported that in *Amaranthus blitum* the megasporangium forms a row of three cells of which the lowest forms the embryo sac. Woodcock (1931) described the development of the seed in *Amaranthus caudatus*. The work on *Digera arvensis* was started in this laboratory in 1932, but due to various reasons it could not be finished earlier. Since then, two papers have already appeared on this plant, one by Naithani (1933) and the other by Joshi and Rao (1934). In both these papers (specially the latter) there appear to be some points that have been misinterpreted and others that have been overlooked. It is to these that we wish to refer in this paper.

#### 2. *Material and Methods.*

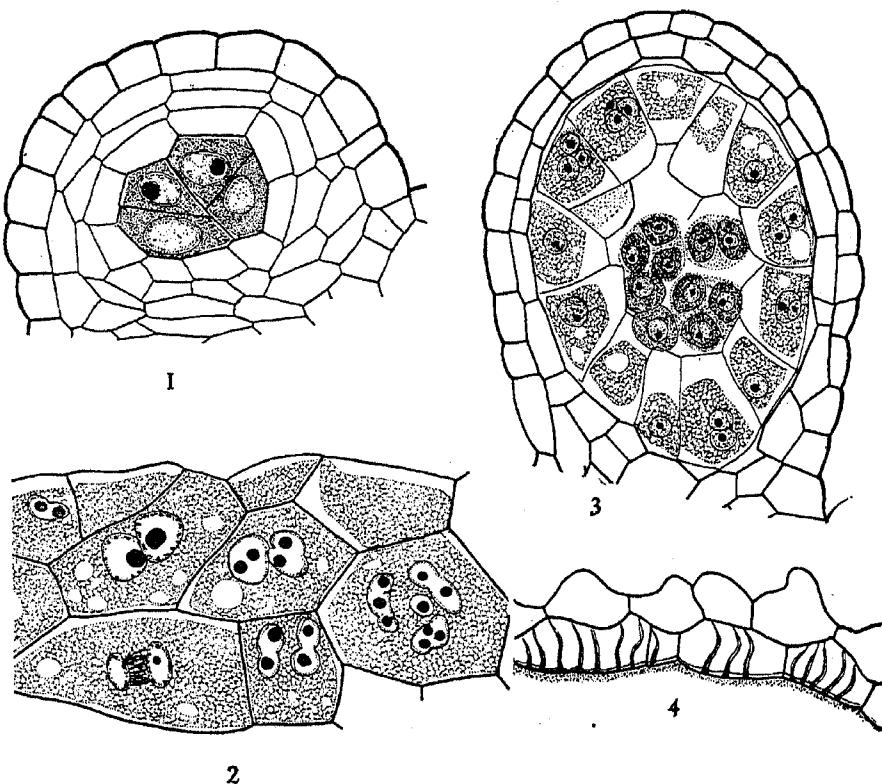
The material was fixed both in Formalin-acetic acid-alcohol and Nawaschin's Fixing fluid. A number of prepared slides and some embedded material were kindly handed over to us by Mr. B. M. Johri and Dr. P. Maheshwari. In staining, the following combinations were used of which the first two gave more satisfactory results:—

(1) Haidenhain's iron-alum haematoxylin, (2) Newton's Gentian violet and iodine, and (3) Safranin and Fast green.

Material of the following additional genera, belonging to this family, has also been fixed and a comparative study of all of them is in progress—*Alternanthera*, *Amaranthus*, *Deeringia* and *Aerua*.

## 3. Microsporogenesis.

A longitudinal section of a young anther shows a linear row of 5-6 archesporial cells. In transverse section each lobe shows only one archesporial cell to begin with. The primary parietal cells, cut off towards the periphery, divide to form an outer endothelial layer and an inner one, which again divides resulting in the middle layer and the tapetum (Fig. 1).



Figs. 1-4.  $\times 840$ . Fig. 1.—T. S. of a young anther lobe showing wall layers and the sporogenous tissue. Fig. 2.—Tapetal cells in tangential section showing the multinucleate condition, lobed appearance of some of the nuclei and a mitosis in one cell. Fig. 3.—The cytoplasm has receded from the inner wall of the tapetal cells. Fig. 4.—The epidermis and endothecium; the latter is distinguishable by the characteristic fibrous thickenings.

The tapetal cells form a complete jacket round the sporogenous mass which now, as mentioned by Joshi and Rao, consists of a plate of 4 or 5 cells in cross section. They are glandular in nature and often bigger than the microspore mother cells. About the time of reduction division the tapetal cells become multinucleate. The two-nucleate condition is the most common but cells with three, four (Figs. 2 and 3) or even five nuclei are met with quite often. Joshi and Rao's statement that "more than two nuclei in a tapetal cell such as have been recorded in *Typha* by Schaffner and in *Hepatica* by

Coulter were never seen", seems to have been based upon insufficient observations. Similar appears to have been the case with Naithani. The first division of the nucleus in any tapetal cell always appears to be mitotic, but we are unable to make any statement about the later divisions. Often there is seen a multinucleolate nucleus with a lobed appearance, which may either be due to the fusion of many nuclei or may be a stage preparatory to amitotic division. Usually the tapetum persists up to the time when the microspores are fully formed, and shrivels up when the pollen grains are in the process of maturation. When the tapetal cells have disappeared altogether, a thin layer consisting of yellowish granules (as seen in sections stained with iron-alum haematoxylin) comes to lie in their place (Fig. 4). This, probably, is the unused material of the tapetal cells. In one solitary instance the tapetal cells were seen to form a periplasmidium in which were embedded the degenerating tri-nucleate pollen grains.

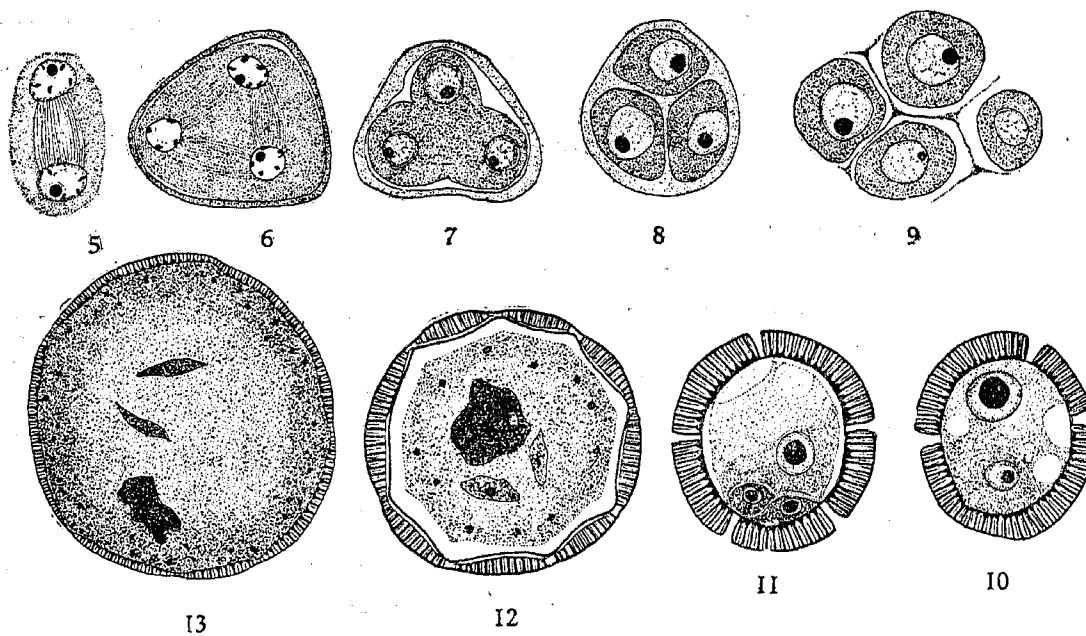
The single middle layer, which lies just outside the tapetum, becomes flattened and crushed and disappears almost completely during the formation of the tetrads (Fig. 3). The hypodermal layer develops into the usual endothecium with characteristic fibrous thickenings (Fig. 4). It is surprising to find it mentioned by Joshi and Rao that the cells "just below the epidermis of the anther do not develop into a fibrous endothelial layer". The only explanation we can give of its supposed absence is that perhaps these authors did not examine sufficiently old stages.

The total number of mother cells in an anther lobe<sup>1</sup> seems to range on an average between 25-30. This point has been confirmed by counting the number of young microspores in an anther lobe and dividing this by four. It is only in rare cases that this number reaches as high as 40, which is believed by Joshi and Rao to be the usual number.

The microspore mother cells now prepare for reduction division. As stated by Joshi and Rao, it is noteworthy that the mother cell walls do not get rounded and separated from one another up to this stage. They still form a more or less compact tissue although the cytoplasm has already rounded up. It is only at the tetrad formation stage that they become free from one another. Joshi and Rao's Plate II, Figs. 9-12 might, however, be taken to imply that this rounding of the mother cells never occurs, not even up to the time the tetrads are formed.

<sup>1</sup> Naithani uses the term pollen chamber for pollen sac. This term has always been used to designate the disintegrated nucellar tip of Gymnosperms and its use for the anther loculus is unfortunate. Farr (1920) also misuses it similarly.

*Heterotypic Division.*—During the prophase of the first reduction division the cytoplasm recedes back from the mother cell wall which begins to show signs of future gelatinisation. During the diakinesis stage we have been able to count the number of chromosomes which corresponds to 6 ( $n$ ) and 12 ( $2n$ ) as given by Joshi and Rao. Metaphase and anaphase have already been closely studied by these authors. In early telophase the two groups of chromosomes lie at opposite poles in the form of flat discs. Later on they expand, become spherical and develop a nuclear membrane. During the period of interkinesis<sup>2</sup> a small nucleolus makes its appearance in each daughter nucleus (Fig. 5). Joshi and Rao (1934, p. 218), on the other hand, completely deny the occurrence of nucleoli in interkinetic stage.



Figs. 5-13.  $\times 1367$ . Fig. 5.—Microspore mother cell; telophase of first reduction. Fig. 6.—Tetra-nucleate stage of the mother cell. Fig. 7.—Furrowing has started and the mother cell wall has become gelatinised. Fig. 8.—Quadripartition is completed and the gelatinous matrix has penetrated in between the microspores. Fig. 9.—The appearance of four radiating walls separating the microspores. Figs. 10-11.—Two- and three-celled pollen grains. Figs. 12-13.—Pollen grains which appear to be in process of degeneration. Note the gradual thinning of the exine with the increase in the size of the pollen grains.

*Homotypic Division.*—Soon after the spindle fibres connecting the two daughter nuclei have disappeared, the nuclear membrane dissolves and the second reduction division begins. The two spindles may lie in various planes

<sup>2</sup> In their paper Joshi and Rao have used the term interphase to denote interkinesis. As a matter of fact, interphase stands for the interval between two mitotic divisions, while the period between two meiotic divisions is known as interkinesis.

inside the mother cell, but the form which results in the tetrahedral arrangement of microspores is the most common. As soon as the four groups of chromosomes have reached their respective poles they become interconnected by means of secondary spindles. The two homotypic spindles persist as such while four secondary ones arise *de novo*. Thus in all there are six spindles which connect every one of the four nuclei of a tetrahedral tetrad with the other three (Fig. 6).

**Cytokinesis.**—On page 219, Joshi and Rao write that "the formation of the pollen grains from the pollen-mother-cell in which the four grand-daughter nuclei are embedded, takes place by furrowing as described by Farr". Unfortunately this paper of Farr (1916) was not available to us, so we cannot say which plant is referred to here, but this much is certain that in the mode of cytokinesis *Digera arvensis* differs a good deal from the condition in *Nicotiana* and offers a better comparison with *Cobaea scandens* (Farr, 1920).

After the heterotypic division no trace of a cell plate is observed. As a matter of fact the achromatic figure is quite homogeneous from one pole to the other. After the tetra-nucleate stage six furrows start, as sharp cutting edges formed by the infolding of the plasma-membrane, just along the equator of each spindle (Fig. 7). The rate of furrowing seems to be quite uniform at all points as is the case in *Cobaea*. In *Nicotiana*, on the other hand, furrowing proceeds at a much greater speed at the four points where three furrows meet, and this is responsible for the appearance of a triangular area in a transversely cut tetra-nucleate cell. The non-existence of this area in the case of *Digera arvensis*, together with the separation of all the microspores at the same time, clearly shows that the rate of furrowing is more or less uniform at all points. Sometimes one comes across a section which shows a greater depression on one side than on the others. This is due to the plane of section, as is very clearly explained by Mrs. W. K. Farr in *Cobaea scandens*. The rate of furrowing appears to be very rapid, for many cases were observed where the furrows have just started and also many where the microspores have just been formed, but the intervening stages were very rare.

About the tetra-nucleate stage the protoplast of the mother cell comes to be surrounded by a mucilaginous wall which sometimes is not seen in sections stained with iron-alum haematoxylin but becomes visible with Fast green. The nature and origin of this wall has attracted much attention during recent years. Farr (1916) considers that this is merely a product of the swelling and gelatinisation of the secondary strata of the mother cell wall and that the thickening is not growth but is "in the nature of an increase in the colloidal dispersity of the wall". Gates (1924), on the other hand, observes that in *Lathraea* this wall is secreted by the cytoplasm in between

the plasma-membrane and the mother cell wall. Castetter (1925) also reports a similar condition in *Melilotus alba*. Beer (1911) seems to have found an intermediate condition in *Ipomoea*, where he distinguishes this wall into two parts, an outer one formed by the gelatinisation of the mother cell wall, and an inner one secreted by the cytoplasm itself (Beer, 1911, Fig. 2). Our observations on *Digera* seem to support Farr's conclusion, for here we have been able to study the gradual transformation of the mother cell wall into a gelatinised sheath. Both Gates and Castetter have emphasised the independence of the original mother cell wall from the gelatinised or special wall. In *Digera* no such independence could be observed.

Farr's idea (1920) that the greater the amount of gelatinisation of the cell wall the greater the width of the furrow, is also supported by the conditions in *Digera*. Here the gelatinous wall is comparatively thin and the furrows are, therefore, correspondingly sharp and narrow. She explains this by saying that a broad wall formed as a result of imbibing more water becomes much softer and so allows a greater amount of change in the form of the protoplasm caused by broader furrows.

Joshi and Rao do not make any mention of the gelatinous wall and their Plate II, Figs. 9-12, might be taken to imply that this gelatinisation never occurs. This becomes still more clear when they write (see p. 219) that "at first the four pollen cells are surrounded by a small layer of cytoplasm of the mother-cell, but this is soon absorbed". A similar case was reported by Kanda (1920, p. 60) who writes that, "In *Verbena angustifolia* there are two different types of tetrad-formation. In the one case the peripheral cytoplasm of the pollen-mother-cell is left over to form a wall for the tetrad, this wall subsequently disintegrating, while in the other case the entire mother cell is utilised in the formation of the tetrad." We consider it more likely that in both *Digera* and *Verbena* the authors are dealing with the gelatinous wall, for it is difficult to imagine how any cytoplasm can remain outside the plasma membrane which had formed the limiting layer of the four pollen cells.

Sometimes it so happens that the tetrads with their gelatinous walls appear to be surrounded by a 4—6-sided skeleton of walls; more often these walls cross over the body of the tetrad. Their nature was discovered only when they were found to be absent in sections passing through the middle of the anther lobe. With the advancing age of the anther the cytoplasm of the tapetal cells recedes from the inner wall leaving a clear space on the inner side (Fig. 3). When a section passes tangentially through these spaces we can occasionally see only the cell boundaries of the tapetal cells. In some cases by chance some of the tetrads come to lie just above or below

these cavities and so appear to be enclosed within definite walls. It is just possible that Joshi and Rao's Figs. 9-12 are illustrative of such skeletons of walls while the real wall has already been gelatinised and may not have been discernible due to inadequate staining methods.

When the microspores are about to be set free, they are occasionally separated from one another by means of radiating wall-like structures (Fig. 9). A similar case has also been reported by Beer (1911) in *Ipomoea*. He regards them as "the first lamellæ which are formed at the conclusion of the division of the mother cell". Mangin (1889) has reported the same in *Althaea rosea*, and he states that they are nitrogenous in nature. We are at present inclined to believe that in *Digera* they are formed by a coming-together process of the gelatinous material.

#### 4. Male Gametophyte.

The separated microspores (about 8-11  $\mu$  in diameter) round up and enlarge. The first division results in the formation of a small generative and a large tube nucleus. As reported by Naithani and also by Joshi and Rao, the former organises itself into a lenticular cell (Fig. 10). By this time the exine has become quite prominent and shows about 10-15 or even more germ pores.

There exists a great variation in the size and structure of the pollen grains which measure from 15-30  $\mu$  in diameter. This increase in size is also accompanied by changes in structure. Pollen grains that are up to about 20  $\mu$  in diameter remain, in general, in a healthy condition and it is only in a few of these cases that the generative nucleus divides while the pollen grain is still inside the anther lobe. We agree with Joshi and Rao that the pollen is usually shed at the bi-nucleate stage. At this time the exine is about  $\frac{1}{6}$ th of the diameter of the entire pollen grain and stands out as a yellowish structure against a bluish intine (Newton's Gentian violet-iodine method). Degenerations seem to be quite common even at this stage and may involve all the pollen grains of an anther lobe. As reported by Favorsky (1928), we also found that the pollen of otherwise normally stained preparations sometimes appeared very dark and that additional destaining resulted in a uniform loss of colour without differentiation. Even in the same preparation some of the pollen grains were absolutely black while others were completely destained. The nuclei of such grains were crescent-shaped and often showed only the nucleolus. Artschwager and Starrett's (1933) Plate II, Figs. B, C and E, clearly illustrate the same condition in *Beta vulgaris*.

In some cases the pollen grains increase in size; the cytoplasm becomes homogeneous and takes a deeper stain, vacuoles disappear, starch grains make

their appearance and the generative nucleus divides to form two spindle-shaped male cells (Figs. 12-13). No definite structure can be made out of the sperm cells and the tube nucleus. All of them are jet-black and the latter shows a lobed appearance prior to fragmentation. On the whole, the general condition of these pollen grains leads us to believe that they are degenerating but this point needs to be confirmed by making some attempts to germinate them in the next flowering season. Billings (1934, Figs. 8-10) also gives three diagrams of a similar type for *Atriplex* and the last of these is regarded by him as illustrating a "mature pollen grain showing complete nuclear disintegration".

The size variation in the mature pollen grains is very striking and all the intermediate stages are met with. We are unable to understand why the degenerating pollen grains should increase in size. Bhargava's Figs. 36-37 (1932) also seem to illustrate this point quite clearly, although he does not make any statement about this in the text. Oksijuk (1927) observed pollen grains of two sizes in his material of *Beta vulgaris*, a large percentage of small grains with an average diameter of  $16.8\mu$ , and a comparatively smaller number with a diameter of  $21.5\mu$ . As no intermediate sizes are found, Oksijuk believes that the two types belong to two different races. This, however, does not seem to explain the condition in *Digera*.

A notable peculiarity has been seen in connection with the fate of the exine. As has been mentioned before it is about  $\frac{1}{6}$ th of the total diameter of a healthy pollen grain at the time of shedding. With the increase in size, and the approach of the conditions which we regard as leading to degeneration, the exine seems to get thinner and thinner, till it becomes very much reduced in completely degenerated pollen. One can hardly avoid the conclusion that during later stages of development the material composing the exine begins to be dissolved away. A more or less similar condition is reported by Brough (1924) in *Styphelia longifolia*, for in this case also the mature pollen grains show a much thinner exine than the uninucleate pollen grains. As an explanation he writes that "the thick stratified walls of the younger microspores represent a reserve store of cellulose which is drawn upon during the rapid development which precedes the production of mature spores."

The two-nucleate condition of the pollen grain, which appears to be the more common one in *Digera*, is of rare occurrence in other Centrospermales. So far it has been reported in *Boerhaavia diffusa* (Maheshwari,<sup>3</sup> 1929), *B. repanda* (Bhargava, 1932) and some members of the Portulacaceæ.

<sup>3</sup> Recently, in some slides prepared by one of my pupils, Mr. B. P. Paliwal, I have seen several three-nucleate pollen grains, but these are all in an advanced state of degeneration.

P. Maheshwari.

5. *Megasporogenesis.*

The archesporium consists of either a single hypodermal archesporial cell, or more frequently a group of 2-5 cells lying just below the nucellar epidermis, but usually only one undergoes the changes necessary to the formation of megasporangia.

The first division of the archesporial cell separates a primary wall cell from the sporogenous cell. The former is always the first to divide. We do not agree with Joshi and Rao when they say that "there seems to be no regularity in the sequence of the division of these two cells". In order to substantiate their point these authors refer to their Plate III, Fig. 6, where the megasporangium mother cell is supposed to have divided first. This seems to us to be incorrect, as in our opinion they are here dealing with a group of two sporogenous cells lying one above the other. That this is the correct interpretation appears to have been admitted at another place even by Joshi and Rao, where they describe the number of archesporial cells in the nucellus (see p. 221).

The megasporangium mother cell increases in size and divides twice to form a linear row of four megasporangia. Sometimes there is a row of only three cells, the upper one of the two dyad cells having remained undivided. Naithani (1933) regards this to be the usual condition, but this is really not so as is also held by Joshi and Rao. In all cases seen by us the chalazal megasporangium<sup>4</sup> functions and the others degenerate very early. In this connection Joshi and Rao mention some very striking exceptions. For instance, they write that (pp. 221-222), "in several cases it appeared that the upper megasporangium of a linear row of 4 would form the embryo-sac (Plate III, Figs. 11-12). In these cases this megasporangium had enlarged very much, and in some cases, it was in the early prophase (Fig. 12)." Even a casual glance at this figure, which is here reproduced as Fig. 14, would reveal that what is regarded by these authors as a functioning megasporangium is really a megasporangium mother cell, mounted on top of an axial row of three potentially sporogenous cells! This is made very clear by the presence of the synizetic knot in this cell. Our Fig. 15 also elucidates the same point quite clearly, although a row of only three cells is seen here.

Further, on p. 222, Joshi and Rao write that two cases were found in which the four megasporangia were seen to be arranged in a more or less tetrahedral and bilateral manner. In fact, both these illustrations, here reproduced as Figs. 16-17 respectively, merely show groups of four sporogenous

<sup>4</sup> Naithani (1933, p. 133) uses the term 'embryo sac mother cell' for the functioning megasporangium. As a matter of fact, this term is usually applied to the megasporangium mother cell.

cells (or "secondary archesporial cells") arranged in different manners. Their form, their structure and the relative size of the nucellus fully justify this interpretation.

#### 6. *Megaspores.*

Joshi and Rao (1934) have again misinterpreted the condition seen in their Plate IV, Fig. 6, here reproduced as Fig. 18. In their own words (pp. 223-224), "Here was found a row of 5 cells which in staining and other features had the characters of spores and were quite distinct from the other cells of the nucellus. The three lower cells possessed resting nuclei while the nuclei of the uppermost cells were in metaphase. The wall between these cells was very thin and at places indistinct and appeared to be in a state of dissolution." As an interpretation they suggest that the lower three cells are non-functional megaspores and that the uppermost functional megaspore "had divided once, but unlike the normal case a wall was formed after this division. Each daughter nucleus was again dividing and an embryo sac was being formed by dissolution of the intervening wall between two upper cells....."

There is scarcely any doubt that this represents a dyad in division mounted on an axial row of three well-differentiated nucellar or potentially sporogenous cells as seen in Fig. 14. The condition of the nucellus as well as the presence of a separating membrane fully justifies this interpretation. An almost parallel case was seen by Bhaduri (1932, Plate I, Fig. 5)<sup>5</sup> in *Solanum melongena*, who has interpreted the structures quite correctly. Our Fig. 20 shows three megaspores mounted on top of four large nucellar cells.

#### 7. *Embryo Sac and Embryo.*

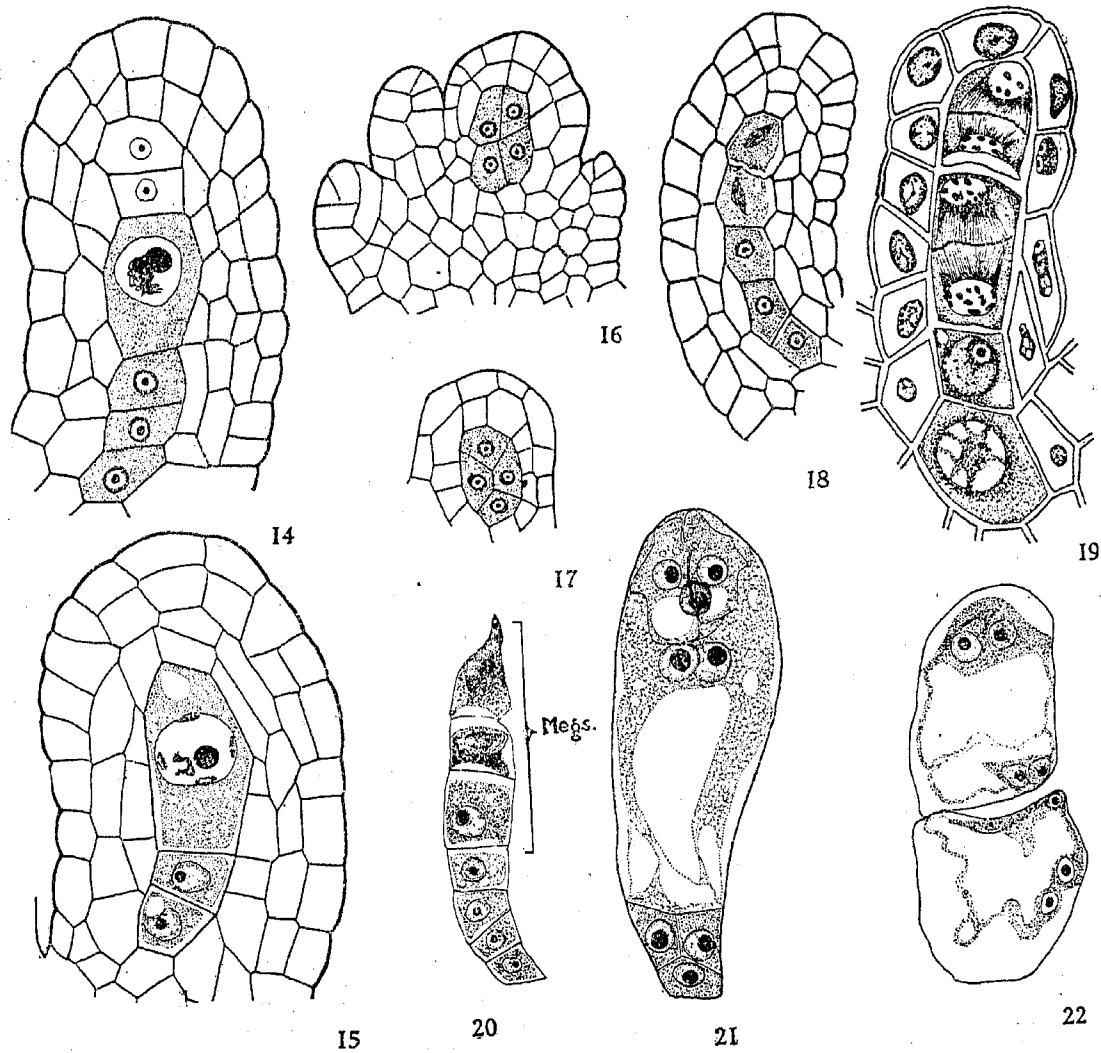
Our observations on the development and organisation of the embryo sac are in accord with those of Naithani as well as of Joshi and Rao. The synergids bear hooks and are somewhat smaller than the egg (Fig. 21).

With regard to their Plate IV, Fig. 7, Joshi and Rao state that the polar nuclei have remained free from each other and that the second male nucleus has fused with one polar nucleus alone. We think it more likely that these are two endosperm nuclei resulting from the first division of the primary endosperm nucleus.

During the later stages of embryo development the inner layer of the inner integument and the outer layer of the outer one become characteristically thickened as has been described by Woodcock in many of the Centrospermales.

<sup>5</sup> This is reproduced here as Fig. 19.

One abnormal case was observed, where a nucellus contained two embryo sacs lying one above the other and each containing four nuclei (Fig. 22). It is difficult to say if these embryo sacs are derived from two megasporangia of



Figs. 14-22. Fig. 14.—(Copied from Joshi and Rao's Plate III, Fig. 12)—Supposed by them to show the enlargement of the uppermost megasporangium, but really a megasporangium mother cell in synizesis. Fig. 15.—A megasporangium mother cell on top of two large nucellar cells.  $\times 840$ . Figs. 16 & 17.—(Copied from Joshi and Rao's Plate III, Figs. 15 & 16)—Interpreted by them as tetrads of megasporangium; for our interpretation, see text. Fig. 18.—(Copied from Joshi and Rao's Plate IV, Fig. 6)—For explanation, see text. Fig. 19.—(Copied from Bhaduri on *Solanum melongena*, 1932)—Showing 2 dyad cells in division on top of two nucellar cells, which simulate in appearance the sporogenous cells. Fig. 20.—An axial row of three megasporangia (Megs.) mounted on top of four nucellar cells. The chalazal megasporangium has enlarged.  $\times 840$ . Fig. 21.—Mature eight-nucleate embryo-sac.  $\times 840$ . Fig. 22.—2 four-nucleate embryo-sacs lying one above the other.  $\times 840$ .

the same tetrad, or they are the products of two different megasporangium mother cells lying one above the other, but the absence of any intervening cells renders the former interpretation more likely.

#### 8. Discussion.

According to Engler and Prantl (1894), the order Centrospermæ consists of the families Chenopodiaceæ, Amaranthaceæ, Nyctaginaceæ, Cyanocrambaceæ (=Thelygonaceæ), Phytolaccaceæ, Aizoaceæ, Portulacaceæ, Basellaceæ and Caryophyllaceæ. Wettstein\* (1924) includes the Cactaceæ also in this order. Hutchinson (1926) breaks up this whole group into two orders:—*Caryophyllales*, consisting of the Elatinaceæ, Caryophyllaceæ, Molluginaceæ, Ficoidaceæ (Aizoaceæ) and Portulacaceæ; and *Chenopodiales*, consisting of the Phytolaccaceæ, Cyanocrambaceæ, Chenopodiaceæ, Batidaceæ, Amaranthaceæ and Basellaceæ.

The families Chenopodiaceæ, Amaranthaceæ, Nyctaginaceæ, Phytolaccaceæ, and Aizoaceæ form a very natural assemblage. The floral plan is simple and uniform. Anatomically they all show an anomalous secondary growth arising through successive development of extrafascicular cambium rings. From the embryological point of view the following features may be regarded as characteristic of them:—

- (a) A typically glandular anther-tapetum, which does not form any periplasmidium.
- (b) A generally three-nucleate condition of the mature pollen.
- (c) Periclinal divisions in the nucellar epidermis.
- (d) The formation of the micropyle by the inner integument only.
- (e) The curved appearance of the embryo sac.

The accompanying chart summarises the more important embryological feature of all the families belonging to the order Centrospermæ.

The position of the Cactaceæ, which was hitherto doubtful, now seems to be somewhere near the Aizoaceæ, while the Cyanocrambaceæ (=Thelygonaceæ) is thought to occupy an isolated position in the order. Further investigations on both of these are necessary before a definite opinion can be ventured.

The remaining 3 families Basellaceæ, Portulacaceæ and Caryophyllaceæ show some important differences in having a more advanced floral organisation and the Phytolaccaceæ, which possesses both simple and complex types of floral structures, seems to connect the two groups in some ways. Although some genera of the Caryophyllaceæ (see Pfeiffer, 1926, p. 23) do show the anomalous secondary growth characteristic of the Chenopodiaceæ, the Portulacaceæ and Basellaceæ do not show this anomalous structure at all.

The latter was originally included in the Chenopodiaceæ (Bentham and Hooker), but the simple structure of its root and stem, without the type of anomaly characteristic of the Chenopodiaceæ, warranted its separation from the latter (Solereder, 1908, pp. 663-664).

In embryological features some further points of difference are noticeable. Periclinal divisions in the nucellar epidermis—a characteristic feature of the Chenopodiaceæ and the families allied to it—have not been observed in any member of the Caryophyllaceæ. Another difference is the frequent presence of a multicellular archesporium in the Caryophyllaceæ, although the Phytolaccaceæ and occasionally some other families of the order also show the same condition to a certain extent. Schnarf (1933, p. 281) regards that in the Caryophyllaceæ the female gametophyte, the endosperm, and the embryo follow quite a peculiar differentiation. In describing cell formation in the endosperm of this family Rocén (1927) distinguishes three types:—

(1) *Silene-type*.—Cell formation occurs in the entire embryo sac as in *Silene* and *Paronycheæ*. The family *Basellaceæ* also shows the same condition. In this respect both these families differ from the Chenopodiaceæ and its allies, where wall-formation is usually restricted to micropylar end.

(2) *Melandrium-type*.—Only the micropylar end becomes cellular as in most of the *Lychnideæ*, *Diantheæ*, *Alsineæ* and *Sperguleæ*. Most of the *Portulacaceæ* show the same condition.

(3) *Heliosperma-type*.—Only a single layer of endosperm cells is found surrounding the hypocotyl of the embryo as in *Heliosperma*, *Stellaria* and *Polycarpeæ* and also some of the *Portulacaceæ*.

It seems that during the past, too much importance has been attached to the curved condition of the embryo seen in all these families.<sup>6</sup> In spite of some obvious similarites between the families included under the *Centrospermales*, we think this order is rather unwieldy and at the same time somewhat heterogeneous. It would perhaps be more convenient to separate this into two groups:—(i) *Centrospermales*, consisting of Chenopodiaceæ, Amaranthaceæ, Phytolaccaceæ, Nyctaginaceæ, Aizoaceæ, Cactaceæ and Cyanocrambaceæ; and (ii) *Caryophyllales*, consisting of Portulacaceæ, Basellaceæ and Caryophyllaceæ. Hutchinson's separation of the Aizoaceæ from the Chenopodiaceæ does not seem to be justifiable.<sup>7</sup>

The above suggestions can only be tentative till further investigations have been made with regard to some of the families considered here, especially

<sup>6</sup> The term 'Curvembreae' is also used for this order.

<sup>7</sup> See Bhargava (1935) for a full discussion of the relationships of the Aizoaceæ and the Nyctaginaceæ.

the Cactaceæ, Aizoaceæ and Cyanocrambaceæ. What we wish to emphasise, however, is that a nearer approach to a natural classification can be obtained from a study of the plants in their entirety, that floral characters alone are not enough for the purpose, and that anatomy and embryology are capable of helping in the solution of taxonomic problems.

#### 9. Summary.

1. Microsporogenesis and the development of the male gametophyte follow the usual course. Each anther lobe contains about 25-30 mother cells. The tapetal cells may contain as many as five nuclei. The endothelial layer develops the usual fibrous thickenings.
2. The haploid and diploid number of chromosomes is 6 and 12 respectively.
3. Cytokinesis takes place by furrowing. The mother cell wall gelatinises at the tetra-nucleate stage or even before it.
4. Great variations in the size of the pollen grains and thickness of the exine are met with. Degeneration is very common. Pollen grains are shed both at two- and three-nucleate stages.
5. In the nucellus there are usually several archesporial cells but normally only one succeeds in forming the embryo sac.
6. A linear tetrad of four megaspores is the rule, but in some cases the upper of the two dyad cells fails to divide, thereby resulting in a linear row of only three cells. In every case the lowest megasporule functions.
7. The mature embryo sac is of the normal 8-nucleate type. The synergids are hooked and the antipodals are ephemeral.
8. It is suggested that the families Portulacaceæ, Basellaceæ and Caryophyllaceæ be separated and put in another order, the Caryophyllales.

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#### LITERATURE CITED.

Artschwager, E. . . . . "Micro and Macrosporogenesis in Sugar beet with special reference to the problem of Incompatibility," *Mem. Hort. Soc. New York*, 1927, 3, 295-297.

Artschwager, E. . . . . "Development of flowers and seed in Sugar beet," *Jour. Agri. Res.*, 1927, 34, 1-25.

Artschwager, E., and Starrett, R. C. . . . . "The time factor in fertilization and embryo development in the Sugar beet," *Jour. Agri. Res.*, 1933, 47, 823-843.

Beer, R. . . . "Studies in spore development," *Ann. Bot.*, 1911, 25, 199-214.

Bhaduri, P. N. . . . "The development of the ovule and embryo sac in *Solanum melongena L.*," *Jour. Ind. Bot. Soc.*, 1932, 11, 202-224.

Bhargava, H. R. . . . "Contribution to the morphology of *Boerhaavia repanda*," *Jour. Ind. Bot. Soc.*, 1932, 11, 303-326.

Bhargava, H. R. . . . "Contribution to the morphology of *Mollugo nudicaulis Lamk.*," *Proc. Ind. Acad. Sci.*, 1934, 1, 271-278.

Bhargava, H. R. . . . "The life-history of *Trianthema monogyna* Linn.," (In the press).

Billings, F. H. . . . "Male gametophyte of *Atriplex hymenelytra*," *Bot. Gaz.*, 1934, 95, 477-484.

Brough, P. . . . "Studies in the Epacridaceæ. I. The life history of *Stypelia longifolia* (R. Br.)", *Proc. Linn. Soc., New South Wales*, 1924, 49, 162-178.

Castetter, E. F. . . . "Studies on comparative cytology of the annual and biennial varieties of *Medicago alba*," *Amer. Jour. Bot.*, 1925, 12, 270-286.

Dahlgren, K. V. O. . . . "Zytologische und embryologische Studien über die Reihen Primulales und Plumbaginales," *Svenska Vetensk. Akad. Handl.*, 1916, 56, 1-80.

Engler, A., and Prantl, K. . . . "Die natürlichen Pflanzenfamilien," III Teil, 1. Hälfte, Leipzig, 1894.

Farr, C. H. . . . "Cytokinesis of the pollen-mother cells of certain dicotyledons," *Mem. New York Bot. Gard.*, 1916, 6, 253-317. (Quoted in Farr, 1918; Gates, 1924.)

Farr, C. H. . . . "Cell division by furrowing in *Magnolia*," *Amer. Jour. Bot.*, 1918, 5, 379-395.

Farr, W. K. . . . "Cell division of the pollen-mother cells of *Cobaea scandens alba*," *Bull. Torrey Bot. Club*, 1920, 47, 325-338.

Favorsky, N. . . . "Materialien zur Biologie und Embryologie der Zuckerrübe," *Trudy Nauch. Inst. Selek.*, 1928, 2, 1-18. (In Russian with German summary.) (Quoted in Artschwager and Starrett, 1933.)

Gates, R. R. . . . "Pollen tetrad wall formation in *Lathraea*," *La Cellule*, 1924, 35, 49-58.

Huber, J. A. . . . "Zur Morphologie von *Mesembrianthemum*," *Bot. Archiv. Mes.*, 1924, 5, 7-25.

Hutchinson, J. . . . "Families of flowering plants. Vol. I., Dicotyledons," London, 1926.

Joshi, A. C., and Rao, C. V. . . . "A contribution to the anatomy, morphology and cytology of the flower of *Digera arvensis* Forsk.," *Jour. Ind. Bot. Soc.*, 1934, 13, 201-236.

Kanda, M. . . . "Field and Laboratory studies of *Verbena*," *Bot. Gaz.*, 1920, 69, 54-71.

Lewis, I. F. . . . "Notes on the development of *Phytolacca decandra*," *John Hopkins Univ. Circ.*, 1905, 178, 34-42.

Maheshwari, P. . . . "Contribution to the morphology of *Boerhaavia diffusa* L.," *Jour. Ind. Bot. Soc.*, 1929, 8, 219-234.

Mangin, L. . . . "Observations sur le développement du pollen," *Bull. Soc. Bot. France*, 1889, 36, 391 (Quoted in Beer, 1911).

Mauritzon, J. . . . "Ein Beitrag zur Embryologie der Phytolaccaceen und Cactaceen," *Botaniska Notiser Lund*, 1934, 111-135.

Naithani, S. P. . . . "A contribution to the morphology of *Digera arvensis*," *Bull. Acad. Sci. U.P.*, 1933, 3, 119-128.

Oksijuk, P. . . . "Entwicklungsgeschichte der Zuckerrübe (*Beta vulgaris*)," *Bull. Jard. Bot. Kieff*, 1927, 5-6, 1-19. (In Russian with German summary.)

Pfeiffer, H. . . . "Das Abnorme Dickenwachstum" in *Handbuch der Pflanzenanatomie*, Band 9, Berlin, 1926.

Rocén, T. . . . "Zur Embryologie der Centrospermen," *Akad. Abhandl. Uppsala*, 1927.

Schnarf, K. . . . "Vergleichende Embryologie der Angiospermen," Berlin, 1931.

Schnarf, K. . . . "Die Bedeutung der Embryologischen Forschung für das natürliche System der Pflanzen," *Biologia Generalis*, 1933, 9, 271-288.

Solereder, H. . . . *Systematic Anatomy of Dicotyledons*, Vols. I and II, Eng. Transl., Oxford, 1908.

Woodcock, E. F. . . . "Morphological studies of the seed of *Alsine media*," *Mich. Acad. Sci. Arts and Letters*, 1926, 6, 397-401.

Woodcock, E. F. . . . "Seed development in *Amaranthus caudatus* L.," *Mich. Acad. Sci. Arts and Letters*, 1931, 15, 173-177.

Woodcock, E. F. . . . "Observations on the morphology of the seed in *Phytolacca*," *Mich. Acad. Sci. Arts and Letters*, 1924, 4, 413-418.

Woodcock, E. F. . . . "Morphology of the seed in *Claytonia virginica*," *Mich. Acad. Sci. Arts and Letters*, 1925, 5, 195-200.

Woodcock, E. F. . . . "Seed development in *Thelygonium cynocrambe* L.," *Mich. Acad. Sci. Arts and Letters*, 1929, 11, 341-346.

Woodcock, E. F. . . . "Morphological studies on the seed of *Mesembrianthemum crystallinum* L.," *Mich. Acad. Sci. Arts and Letters*, 1930, 13, 221-225.

\* The papers marked with an asterisk were not available to us.