THE DEVELOPMENT OF OVULE AND EMBRYO SAC IN *SOLANUM MELONGENA* L.

BY

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The Family Solanaceae, one of the Metachlamydeae, contains more than seventeen hundred species. Due to the large number, the wide range of distribution, and the economic importance of some of the species, quite a large amount of cytological and genetical investigation has been carried out in this family. One of the most interesting work in this family has been done by Blakeslee, Belling, and Farnham who in a series of papers (2), (3), (4), published the cytology and genetics of the polyploid forms of *Datura Stramonium*, and some of its hybrids. Clausen and Goodspeed (5) also studied the cytology and genetics of *Nicotiana*, as well as some of its interspecific hybrids. Kostoff (22) quite recently studied the microspore formation in Capsicum, and Ferguson (15) reported a new type of fertilisation in her work on the cytology and genetics of *Petunia*. Winkler quite early in a series of papers (36), (37) gave an account of his investigation on graft hybrids in the genus *Solanum*. He fairly dealt with the morphological and anatomical differences of the "heteroploid" plants. Jorgensen (20) has recently extended Winkler's investigations and studied the cytology of the artificially-produced heteroploid plants of *Solanum nigrum* and *Lycopersicum esculentum*. Lesley (23) worked out the cytology of both diploid and triploid forms of *Lycopersicum esculentum*. Kojima (21) has studied meiosis in the microspore formation of two different races of *S. melongena* and counted the chromosome numbers in twenty-one different races of the same species.

The study of the female gametophyte in Angiosperms began towards the end of the nineteenth century. The work up to 1903 has been summarised by Coulter and Chamberlain (8). A resume of later work up to 1923 has been given by Rutgers (28). A general review of the whole subject has been made quite recently by Schurhoff (32) and also by Schnarf (31), and need not be repeated here.

The first cytological investigation on the development of the female gametophyte in Solanaceae is due to Guignard (19), who worked out the embryosac development in *Nicotiana tabacum* and *Cestrum splendens,* and concluded that the embryo sac development in these plants follows the normal type. Soueges (33), Palm (27), and Svenson (34) working respectively with *Atropa belladona, Nicotiana 'Delitabak',* and *Hyoscyamus niger* came to the same conclusion as Guignard. But Nanetti (26) working with *Solanum muricatum* found that the development of the female gametophyte in this plant was after the *'Lilium type'*. The fact became more convincing when Young (39) stated that in *S. tuberosum* the archesporial cell itself functioned as the megaspore mother-cell and by three successive divisions gave rise to the normal eight-nucleate embryo sac, the first division being a reduction division. Banerji (1) working in this laboratory found normal type of embryosac development in *Capsicum annum.* Cooper (7) in a recent paper has shown that the development of female gametophyte in *Lycopersicum esculentum* is of the normal type.

Material and Methods.

Material for this investigation was obtained from plants grown in the college experimental garden. Fixation of material was always done in the field on bright days. Ovaries were carefully dissected out from flower buds in all stages of development and fixed immediately. Best results were obtained from materials fixed between 12 noon and 4 p.m. To facilitate penetration of the fixing fluid, the ovaries were trimmed and in some cases cut transversely or longitudinally into small pieces before fixation.

A number of fixing fluids with different active principles were used but very satisfactory results were obtained from material fixed in Allen's modified Bouin's fluid. Licent's fluid, and mercuric chloride fixative with the following composition, gave good results:—

Corrosive sublimate crystals	5 gms.
Glacial acetic acid	5 cc.
Picric acid (Sat. soln. in 50 per cent alcl.) ...	100 cc.
Urea crystals	1 gm.

This fixing fluid was heated to 40° C. before use.

The material fixed in Bouin's fluid was allowed to remain in the fixing fluid for 24 hours. It was then run up in the course of an hour to 60% alcohol saturated with lithium carbonate to remove the green colour produced by the fixing fluid. It was then

dehydrated in the usual way. Material fixed in mercurie chloride fixative was allowed to remain in the fluid for three to four hours, and then washed repeatedly in 50% alcohol with four changes, at six hours interval each. To ensure complete removal of mercuric chloride from the tissue, iodine solution was added drop by drop to the alcohol till it became permanently reddish brown. It was then dehydrated as usual.

The material was cleared either in xylol or in cedarwood oil. The latter was found to be much satisfactory as it saves time, and makes the material less brittle. The material was then embedded in paraffin and sections were cut 6, 8, 10, and 12 μ thick to study the various stages.

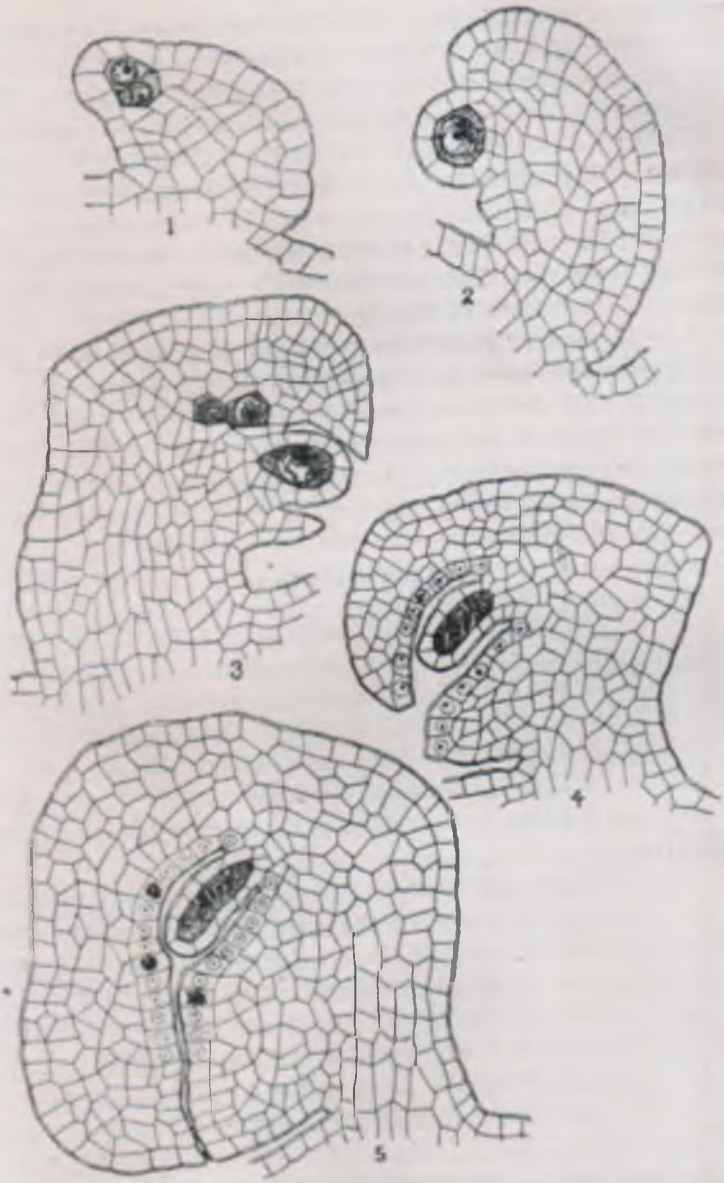
Haidenhain's iron-alum haematoxylin with or without a counter stain was chiefly used for staining. Flemming's triple stain, and Breinl were also tried.

Development of Ovule.

In the initial stages of the development of the ovule, the epidermis of the placentas preserves quite a uniform surface. The cells of the epidermis and three or four layers of sub-epidermal cells soon differentiate out from the rest of the placental tissue. The cytoplasm of these cells is very dense, and each cell contains a conspicuous nucleus which almost fills the entire cavity of the cell. These cells retain the stain more tenaciously than the rest. The ovule initials differentiate out in groups from the sub-epidermal tissue and divide at first only in one direction. The epidermal cells divide anticlinally to keep pace with the protruding ovule initials. The cells lying between the group of ovule initials being unable to keep pace with the actively growing tissue become compressed and distorted. At this stage the placentas loose their evenness, and become wavy in outline, and the identity of the numerous ovules could be made out as blunt papillate processes on the placentas. The ovule initials now divide in all directions, but the growth on one side becomes more vigorous than the other. The papillae soon become somewhat pointed, with the point directed laterally. The archesporial cell differentiates out at this stage, but the funiculus remains short and thick. (Text-Fig. I. i).

Development of the Integumental Tissue.

The primordia of the integumental tissue is first noted after the differentiation of the archesporial cells. The integument first differentiates out as an annular out-growth from the base of the



Text-Fig. I. (i) Early development of ovule, showing two archesporial cells, one above the other. (ii) Development of the megaspore mother cell and the origin of the integument. (iii) Further development of the megaspore mother cell and the integument. Note the abnormal origin of two archesporial cells. (iv) A later stage showing the further development of the integument and the differentiation of the tapetal tissue. (v) The fully developed integument with differentiated tapetal cells. Meiosis is completed and a normal linear tetrad is formed. × 650 (Reduced three-fourth of its original size).

nucellar tissue, and in longitudinal sections appears as two notches. The growth of the ovule soon becomes more rapid on one side, and as a result, the ovule becomes bent (Text-Fig. I. ii). Before the archesporial cell completes the meiotic division, the integumental tissue grows actively and almost overtops the enlarged megaspore mother cell covered by single layer of nucellar tissue (Text-Fig. I. iii). After the formation of the megaspores, the ovules curve further towards the placenta, ultimately coming close to the stalk of the ovule. This gives the ovule an anatropous form, but strictly, it is not so. (Text-Fig. iv, v).

As in most of the plants belonging to Sympetalæ, only one integument is present in this case. This integument is three to four cells thick in the earlier stages of development, but is composed of five or six cells when fully developed. It almost covers the deeply seated embryo sac leaving only a curved narrow passage. (Text-Fig. I. v).

Archesporium

The archesporial cell differentiates out in the hypodermis from a group of sub-epidermal cells. This differentiation takes place before the origin of the integumental primordium, and after the ovule has slightly curved laterally. By this time the microspore mother cells are differentiated in the anthers. The archesporial cell is distinguished from the rest by its bigger size, conspicuous nucleus and relatively dense cytoplasm. (Plate I, Fig. 1).

Besides the general mode of development of the archesporium, variations in its origin and development have been noted. One of the commonest variations is the development of two archesporial cells side by side in the hypodermis. (Plate I, Fig. 2). Sometimes the subhypodermal cell just below the archesporial cell also differentiates out as an archesporial cell. (Text-Fig. I. i). Besides these, three equally developed archesporial cells have been observed to be arranged in a linear direction, this suggests, as also pointed out by Young (39), a case of further division of the archesporial cells. Not infrequently, however, it has been observed that some of the cells of the innermost layer of the integument become differentiated from the rest. (Text-Fig. I. iii). These variations in the development of the megaspore mother cells lead one to believe that all the cells generally included under the group "ovule initials" are potentially sporogenous.

Degeneration of the Functionless Megaspores and the Development of the Embryo Sac.

The enlarged megaspore mother cell by two successive divisions gives rise to a normal linear tetrad of four megaspores (Plate I, Figs. 4, 5). After both the divisions, leading to the formation of the tetrad, the separation of the daughter nuclei by walls is quite evident. The four megaspores when first formed are all alike, with conspicuous nucleus and big deeply stained nucleolus (Plate I, Fig. 6). The resting stage is of a very short duration. The chalazal megaspore soon enlarges and the cytoplasm becomes very much vacuolated, the other three megaspores, however, show signs of degeneration. Generally the micropylar megaspore degenerates first, but some times the second or the third megaspore, from the micropylar end has been observed to degenerate first (Plate II, Figs. 9, 10). The three disintegrated megaspores form a cap over the functioning one and in sections appear as crescents, triangles, or irregular deeply stained masses (Plate I, Figs. 7, 8). In some ovules the third megaspore (from the micropylar end), as well as the chalazal megaspore, have been observed to develop side by side (Plate II, Fig. II). In some preparations more than one embryo sac in the two or four-nucleate stage have been observed in the same ovule, but in no case two mature embryo sacs were noted in the same ovule.

The chalazal megaspore soon enters into the prophasic stage, and divides into two daughter nuclei forming the bi-nucleate embryo sac. They remain close together for a time, but soon the embryo sac begins to enlarge and they move to opposite poles. The cytoplasm becomes very much vacuolated and the two nuclei remain connected by cytoplasmic strands (Plate II, Figs. 12, 13). By two successive divisions the bi-nucleate embryo sac develops into the four-nucleate and later on to the eight-nucleate stage (Plates II and III, Figs. 14, 15, 16); four nuclei being arranged at the chalazal and four at the micropylar end of the embryo sac.

The Mature Embryo Sac.

The mature embryo sac is typical of angiosperms with two synergids and egg at the micropylar end of the embryo sac, two polar nuclei in the centre, and three antipodals at the chalazal end (Plate III, Fig. 17). The synergids appear as pear-shaped cells having long and acute beaks, with a very conspicuous and big vacuole at the base of each. The nucleus which is situated just above the vacuole fills the entire width of the cell (Plate III,

Figs. 18, 19). No filiform apparatus could be made out even with differential staining. The egg nucleus is comparatively smaller in size than the polar, and has dense cytoplasm and a deeply stained nucleolus. The two polar nuclei migrate from the two poles, meet at the centre, or sometimes at one side of the embryo sac. They lie side by side for some time, but soon the wall between them disappears, and the two nuclei ultimately fuse with each other forming the primary endosperm nucleus (Plate III, Fig. 20, a, b, c, d). The three lens-shaped antipodals lie close together filling the chalazal groove of the embryo sac. They are separated from each other by cytoplasmic walls, and appear as distinct cells. They degenerate very soon and all traces are lost long before the time of fertilisation.

The Tapetal Tissue

The presence of a differentiated tapetal tissue covering the female gametophyte is of rare occurrence, as compared to its constant presence in the microsporangium of flowering plants. Evans (13) in *Penistemon Secundiflorus* observed a nutritive layer covering the embryo sac. Schertz (30) has also observed similar tapetal layer lining the embryo sac in *Scrophularia Marylandica* and he has shown that the tapetal layer appears first as a ring of cells surrounding the nucellus which by subsequent anticlinal divisions forms the nutritive tissue. Young (39) in *Solanum tuberosum* has shown that the innermost layer of the integument differentiates and forms a nutritive layer. In the present investigation it has been observed that along with the development of the female gametophyte the single layer of nucellar tissue degenerates. But, by this time, the innermost layer of cells of the integument surrounding the embryo sac differentiates as a layer of nutritive cells. They extend laterally from the micropylar to the chalazal end of the embryo sac.

Mitotic divisions of the nuclei of the tapetal cells leading to the bi-nucleate condition have been observed by several investigators in the microsporangium of various flowering plants. Duggar (12) observed mitotic divisions of the tapetal cells in microsporangium of *Bignonia venusta*. Maheswari (24) also observed similar mitotic divisions in *Boerhaavia diffusa*. Cooper (6) has shown that the formation of bi-nucleate tapetal cells in *Bugnvillaea glabra* is accompanied by mitotic divisions of the nuclei followed by non-formation of cell plates between the daughter nuclei. In the present investigation also, some of the tapetal cells bordering

the embryo sac have been observed in different stages of karyokinetic division, but the nuclei formed have not been observed to develop into separate cells. The tapetal cells are elongated with their long axes perpendicular to the embryo sac, and unlike the cells of the integumental tissue have dense and granular cytoplasm (Plate II, Figs. 14, 15 and Text-Fig. I. iv, v).

Meiosis in the Megaspore Mother Cell.

Resting nucleus of megaspore mother cell:—The megaspore mother cell before it enters into meiotic prophase, undergoes a short period of rest. The cytoplasm is granular and is surrounded by a delicate cell wall. The nucleus generally occupies a central position, and covers almost the entire width of the cell. It is spherical in outline. The nuclear membrane is very fine and the nucleus is surrounded by a dense zone of cytoplasm. The nuclear reticulum is continuous with the membrane and is more or less peripheral. The staining power of the reticulum is feeble and no outline of it can be traced. It appears as a cloudy granular mass with very few deeply stained bodies scattered irregularly over it. The nucleolus is always deeply stained, and lies in a central position. Generally there is only one nucleolus, but in some preparations two or three smaller bodies, probably chromatic in nature, have also been noted. There is always a clear perinucleolar zone, which is interrupted by few faint radiating strips from the nucleolus inside the reticulum. The nucleolus is spherical and comparatively bigger in size than found in the subsequent meiotic stages (Plate IV, Fig. 22). There is sometimes a chromatic protuberance from the nucleolus which persists in some cases up to late diakinesis.

Early prophase:—The first sign of the activity of the nucleus undergoing division can be seen on the reticulum. Small chromatic granules appear on the reticulum which fuse and become bigger. The reticulum retains the stain more tenaciously at some places and the linin connections become prominent as fine irregular threads. The nucleus enlarges in size and the nucleolus becomes smaller in dimension (Plate IV, Fig. 23). The fine connections of linin resolve into definite strands and the beaded chromatic bodies lie strung together by the linin threads. The linin threads bearing the beaded chromatic bodies have been found at some places to run parallel to one another and some times fusing at places. As a result the linin strands appear markedly alveolized. (Plate IV, Fig. 24). The nuclear cavity soon be-

comes filled with a dense tangle of leptotene threads forming a continuous spireme. The individual leptotene threads cannot be followed throughout their entire length and there are marked swellings at the point of intersections. The chromatic protrusion of the nucleolus observed in the resting stage was also evident in certain preparations showing the above stages (Plate IV, Figs. 25, 26, 27).

The first contraction—Synizesis:—The first indication of contraction can be seen in the leptotene threads, which leave the periphery at certain points and begin to move towards one side of the nucleus (Plate IV, Figs. 28, 29), the nucleolus remaining within the tangle of threads. The threads now show a certain degree of polarity, all moving towards one side. In some preparations it has been observed that while most of the threads moved towards one side forming a more or less compact tangle of threads, one or two threads still remained attached to the periphery as free ends or loops. (Plate IV, Fig. 30). The threads enclosing the nucleolus remain contracted for some time and is always to be found on one side of the nucleus. The contraction observed here during synizesis does not appear to be "tight knot" as has been described by many authors for other plants, but, the continuity of the thread can be traced up to a certain length (Plate IV, Fig. 31). Aggregation of chromatic substance, and the contraction of the same within the nucleus has not been observed in this material.

Hollow spireme stage:—The synaptic knot unravels itself and the spireme emerges as distinct loops of variable lengths. The spireme appears at places to be more or less ribbon-like with the chromatic beads remaining strung together (Plate IV, Fig. 32); at certain places it appears to be homogeneous, while at others marked alveolization and consequent fission has been observed. This fission observed in the lengths of this spireme is homologous to that observed in the early prophase. The spireme at this stage is univalent in nature, as also will be evident from its subsequent behaviour. The exact number of the loops thrown out during the opening out of the spireme could not be made out accurately, though their number was never found to exceed the haploid number of chromosomes, but almost approaching the same. In this case, therefore, the two arms of a loop represent two univalent somatic chromosomes attached end to end, which would ultimately form a bivalent heterotype chromosome. (Plate IV, Fig. 33). The spireme then fully unravels itself, completely filling the

entire cavity of the nucleus, and the nucleolus is seen again at the centre. The looping of the continuous spireme is very beautifully represented in this material, although anastomoses take place at certain places due to the comparatively small volume of the nucleus. The spireme is very uniform and thread-like in appearance (Plate V, Figs. 34, 35).

The spireme then thickens considerably and the alveolisation observed in early stages becomes completely obliterated. The pachytene thread is continuous and stains very brightly. It appears as a coiled rope (Plate V, Fig. 37).

Second contraction:—The pairing of the homologous lengths of the spireme which is completed during the second contraction, starts quite early and is not a sudden process. Plate V, Fig. 36 shows early pairing of the univalent lengths of the loops at one end, while at the other end they diverge widely.

Typical second contraction, where pairing is complete has not been observed during this investigation, but a stage leading to the second contraction, where the loops after leaving the periphery are going towards the centre has been observed (Plate V, Fig. 38). The next stage observed was where the threads become more condensed and are thrown out again as loops of variable forms around the nucleolus, the paired univalent lengths of a loop splitting from each other. The pairing of the univalents to form bivalent threads can be still seen at certain places (Plate V, Fig. 39). At the place where they remain attached there is always a marked swelling (Plate V, Fig. 40). The looping of the univalent lengths of the spireme, and the subsequent lateral pairing of the two arms of a loop forming the bivalent chromosomes is a clear evidence of the telosynaptic mode of conjugation.

Formation of bivalent chromosomes:—Soon the thread breaks up into twelve segments, each consisting of two univalents attached end to end. They are found to be of various forms depending on the approximation of the two univalents, and lie scattered throughout the nuclear cavity. They are generally of the form of "V", but sometimes the two arms of a "V" may cross over each other and meet again giving distinct figures of "8", or some times they become interlaced with each other (Plate V, Figs. 41, 42). In some preparations it has been observed that the two arms break apart completely and remain side by side in pairs. These bivalent segments remain still attached by fine thread-like connections, which become obscure in the subsequent stages. (Plate V, Fig. 43).

Diakinesis:—The bivalent chromosomes undergo further condensation and become thickened and stain brilliantly. Each univalent chromosome of a bivalent pair has a central constriction and is more or less bean-shaped. The heterotype chromosomes are now arranged at the periphery of the nucleus, though one or two may be seen at the centre. The nucleolus can be still found with its chromatic protuberance and stains equally bright. (Plate V, Fig. 44). The two short and thick univalent homologous chromosomes of a bivalent pair remain joined with each other by faintly staining strands. This is very conspicuous in some preparations, while in others, no trace of it can be found, due probably, to intense condensation of the chromosomes. Some of these strands become visible again during early anaphase of the first division in the form of "bonds" between the chromosomes. But later on they are drawn into the body of the chromosomes.

Metaphase:—Diakinesis is terminated by the complete disappearance of the nuclear membrane and the nucleolus. The bivalent chromosomes condense still further and become rounded. They stain very deeply with haematoxylin at this stage and are arranged as a plate across the equatorial region of the achromatic figure; the univalent chromosomes of the bivalent pair can still be recognised though not very distinctly (Plate VI, Fig. 45). The spindle appears to be narrow with sharp ends and occupies more than half the length of the megaspore mother cell. Viewed laterally the bivalent chromosomes which are aggregated on the equatorial region of the spindle appear to lose their individuality and look like a clump of stained mass (Plate VI, Fig. 46). In polar views of equatorial plates, however, twelve bivalent chromosomes almost all of equal size, and form, could be made out (Plate VI, Fig. 47). Stout spindle fibre attachments of the chromosomes appear to be the characteristic feature in this material, as it has been observed in material fixed with different fixatives. As has already been observed, each univalent chromosome of a bivalent pair still shows a central construction.

Anaphase:—At the anaphase the disjunction of the bivalent chromosomes takes place. The bivalent chromosomes arranged on the equatorial plate of the spindle can be recognised in early anaphase. At an early stage of the separation of the univalent chromosomes, the attachment between them, which was observed during the metaphase, now becomes quite evident. It consists of a thick single or two thinner bonds (Plate VI, Fig. 48). As the univalents move apart, the bonds between each pair break and

some times they seem to be attached to their respective homologues by tail-like attenuated projections (Plate VI, Fig. 49). Soon the univalents round off, and they appear all alike as they move towards the poles. The univalent chromosomes move apart to either poles as two plates in parallel planes (Plate VI, Fig. 50). In a polar view of this stage, the two plates could be seen in the same section, but in different foci. (Plate VI, Fig. 51). At a later stage the chromosomes become angular in outline and the homotypic split of the second division suddenly becomes evident (Plate VI, Fig. 52, a, b).

Telophase:—The univalents now typically dyad in nature, arrive at the poles simultaneously. The spindle by this time grows wider and a faint zigzag cell plate is formed on the equatorial region of the achromatic figure (Plate VI, Fig. 53). The nuclear membrane now gradually becomes evident, and the chromosomes come close together, and become connected with each other. The spindle becomes barrel-shaped and the cell plate straightens. The spindle is then gradually obliterated and a cell wall is established between the daughter nuclei.

Interkinesis:—In the interkinetic stage, the nucleolus reappears again, but takes the stain very feebly. The nuclear cavity is seen to contain clumps of chromatic elements which are connected by fine linin threads (Plate VI, Fig. 54). This stage is of a very short duration and passes quickly to the homotypic division.

Homotypic division:—The prophasic changes prior to the second division pass away very quickly and the nuclei soon enter into the metaphase stage. As in the heterotypic division a polar view of the equatorial plate reveals the haploid number of chromosomes which appear to be all alike in form and size. The metaphasic spindles were found to lie at right angles, obliquely, or at various angles with each other. They are comparatively smaller in size, and are not so sharp as observed in the heterotypic divisions. The chromosomes as they move apart to the poles are small and round. They do not always move in parallel planes as was noticed in the first division. On arriving at the poles they come close together and form a compact chromatic mass. At this stage the identity of the individual chromosomes is lost. The spindles by this time become broader, and fine cell plates make their appearance in the equatorial region. By this time the nuclei become reconstituted and a nuclear membrane is formed around each of them. By the subsequent differentiation of the cell plates four megaspores are formed which are arranged in a linear manner. (Plate I, Figs. 4, 5).

Mitosis in the Embryo Sac.

As has already been mentioned generally the chalazal megaspore of the tetrad functions, while the others degenerate. The chalazal megaspore soon begins to enlarge and the cytoplasm which was previously very dense, shows signs of vacuolation.

The resting nucleus of the megaspore has a very big and deeply stained spherical nucleolus at the centre, with faintly staining granular peripheral reticulum. The nucleolus is bounded by a clear zone. The resting stage appears to be similar to that observed in megaspore mother cells.

The early prophase is recognised by the appearance of chromatic bodies on the linin strands, which gradually become broader. The nucleolus by this time becomes appreciably smaller in size: no direct budding has been observed. Soon the nuclear cavity becomes filled with a dense tangle of spireme, (Plate VI, Fig. 55), which undergo condensation and ultimately breaks into slender, curved chromosomes, which resemble the somatic chromosomes. (Plate VI, Fig. 57).

The chromosomes are arranged at the equatorial region of the short barrel-shaped spindle and lie interwoven with each other forming a compact mass. A polar view of an equatorial plate, however, shows twelve haploid chromosomes which are very short and thick and are somewhat "V"-shaped (Plate VI, Fig. 56). At a side view in early anaphase, the chromosomes as they move to the poles, appear as short, thick rods. On arriving at the poles the daughter nuclei are reconstituted but no wall formation takes place.

As all the aforesaid stages are of very short duration, and are very difficult to obtain, only a general account of the mitotic division in the embryo sac has been presented.

Discussion.

Embryo sac development:—Early investigators found normal type of development of the embryo sac in Solanaceae. Nanetti (26) and Young (39), however, observed "Lilium type" of development in *Solanum muricatum* and in *Solanum tuberosum* respectively. The question naturally arises whether "Lilium type" of development of the female gametophyte is a general feature in the genus Solanum, or whether it is open to question? In the present investigation in *Solanum melongena*, it has been clearly shown that the development of the female gametophyte follows the "normal type" of development. It is interesting to note in

this connection that a "normal type" of development of the female gametophyte has also been observed in *Solanum nigrum;* a detail account of which will be given in a separate paper. The above facts indicate that "normal type" of development of female gametophyte is a common feature in the genus Solanum, and Nanetti's and Young's works require corroboration before they could be accepted.

Archesporium:—The presence of more than one archesporial cells in the same ovule is not of infrequent occurrence in Dicotyledons. It was thought before, that an archesporium consisting of more than one cell is a primitive feature in Angiosperms, and is only present in groups of plants such as Amentiferae, Ranunculaceae, Rosaceae, etc. This view has, however, been abandoned later on. In Solanaceae, more than one archesporial cell in the same ovule has not been observed up till now, excepting in *Solanum tuberosum,* where Young (39) has observed two, and in a few cases three differentiated archesporial cells. In the present investigation, however, beside the occurrence of two or three hypodermal and sub-hypodermal archesporial cells, the development of some of the permanently sterile nucellar cells into archesporial cells (Plate I, Fig. 5), has also been observed. It will be further interesting to note, that in one case, one of the nucellar cells from the chalazal end of the embryo sac has been observed to develop into a linear tetrad of four megaspores (Plate III, Fig. 21).

Female gametophyte:—The development of two embryo sacs in the two or four-nucleate stages is also an abnormal feature observed in this plant. The occurrence of two embryo sacs in the same ovule has been observed by Young (38) in *Solanum tuberosum.* He believes that this abnormal condition is doubtless "a case of proliferation or doubling, analogous to the hypertrophied condition known as fasciation some times observed in stems and other organs". In *Solanum melongena,* we are inclined to believe that the development of two embryo sacs in the same ovule is due to the activities of any of the other megaspores besides the normal functioning one.

Nucleolar protrusion:—Nucleolar protrusion as has already been described is a very characteristic feature of the megaspore mother cells in *Solanum melongena.* The sudden reduction in volume of the nucleolus entering the prophasic stage, and the persistence of these nucleolar protrusions upto late diakinesis, is suggestive of a flow of nucleolar substance to the developing

spireme. Similar nucleolar protrusions have been observed by many cytologists in a large number of plants. Various theories have been advanced for the phenomenon. Gates and Latter (18) are of opinion that the small papillae from the nucleolus and its distinct connection with the spireme is directly responsible for the elaboration of chromatin, and its subsequent transference to the spireme. Fikry (16) in a recent paper has reviewed the subject exhaustively and it need not be repeated here.

Perinucleolar zone:—In the resting stage of the megaspore mother cell, there is always to be seen a clear perinucleolar zone. McClung (25) is of opinion that there cannot be any clear space surrounding the nucleolus in a well-fixed material, and that the space in question is due to the imperfect fixation of the karyolymph. From the results obtained in *S. melongena* it is very difficult to agree with McClung. The perinucleolar zone persists equally well in preparations fixed with different fixatives. Besides, it has been noted in nuclei of the megaspores as well as in nuclei undergoing meiotic divisions.

Meiosis:—The preceding account of the mode of chromosome reduction follows the telosynaptic interpretation of Meiosis as advanced by Digby (9, 10, 11) and also confirms the view of Kojima (22) who has already pointed out the probability of telosynaptic mode of meiosis in the microspore formation in *S. melongena*, L.

The telosynaptic interpretation of meiosis was first propounded by Farmer and Moore (14) and subsequently supported by a number of investigators. As against the telosynaptic scheme, the parasynaptic interpretation was originally propounded by Winiwater (35) and subsequently supported by Gregoire, and others. Whatever may be the method of pairing of the univalent chromosomes to form the bivalent heterotype chromosomes, the difference between the two interpretations as to the mode of chromosome reduction is, whether the split in the early prophase represent the actual pairing of parental chromosomes as expounded by the parasynaptic theory, or, they represent the closing of the previous telophasic split as advocated by the adherents of the telosynaptic theory. Digby has shown in *Galtonia* (9) and *Osmunda* (11) that the split observed in leptonema stage is homologous with that observed in the premeiotic and somatic divisions, and such study alone showing the relationship of the spireme with that of the premeiotic and mitotic divisions can determine whether the process of chromosome reduction follows the telosynaptic scheme or not.

The explanation becomes easier where no split whatsoever can be made out in the lengths of the developing spireme. In such cases as has been reported by Santos (29), Gates and Rees (17) and others, besides the absence of the split, the heterotype chromosomes are formed by the approximation and condensation of the two univalent arms of a loop, so that the telosynaptic mode of chromosome conjugation becomes quite evident.

In the present observation in *S. melongena,* the parallelism observed in the lengths of the spireme during early prophase is not a true pairing of univalent homologous chromosomes but represent the reassociation of longitudinal halves of somatic chromosomes. Besides the other evidences already advanced before, the suggestion of a telosynaptic mode of chromosome conjugation is evident by the fact, that similar parallelism in the lengths of the spireme has been observed in the nuclei of the megaspore undergoing mitotic division, where no question of pairing of parental chromosomes can arise (Plate VI, Fig. 55). It has also been pointed out that the univalent spireme breaks up into gametic number of loops after the second contraction, the two arms of which come close to each other and condense at the same time, to form the bivalent heterotype chromosomes.

Summary.

1. The ovule initials differentiate as groups of cells from the differentiated sub-epidermal layer.

2. The single integument develops after the differentiation of the archesporium and when fully developed almost covers the deeply seated mature embryo sac.

3. The archesporial cell originates in the hypodermis and functions as the megaspore mother cell. In some preparations more than one archesporial cells were observed.

4. By two divisions the megaspore mother cell gives rise to a linear tetrad of four megaspores. The chalazal megaspore develops and forms a normal eight-nucleate embryo sac while the other three degenerate.

5. The antipodals which appear to be lens-shaped cells degenerate before fertilization.

6. The embryo sac is jacketed by a single layer of tapetal cells, some of which are binucleate.

7. During the meiotic prophase of the megaspore mother cell alveolization of the linin thread is quite conspicuous.

Very tight contraction of the thread was not observed during synizesis. The spireme comes out of synapsis in loops and is continuous. The longitudinal fission in the univalent spireme is homologous with that found in the early prophase; univalent chromosomes remain attached end to end forming the loops. The pairing of the univalent chromosomes begins earlier and is completed during the second contraction. The univalent chromosome of a bivalent pair begin to split apart as they come out of second contraction. Heterotype chromosomes are formed by the segmentation and condensation of these univalent pairs attached end to end. The univalent chromosomes of a bivalent pair remain attached to each other by stainable material which appear during early anaphase as bonds between univalent chromosomes.

8. Homotypic split in the univalent chromosomes reappear suddenly during the anaphase of the heterotypic divisions.

9. Chromatic protrusion of the nucleolus is noted from early prophase to late diakinesis, in the nuclei of the megaspore mother cells.

10. The twelve haploid chromosomes found in the mitotic division in the embryo sac are curved like the somatic chromosomes. The diploid chromosome number determined from somatic cells has been found to be twenty-four.

In conclusion, I take the opportunity of expressing my gratitude to Mr. I. Banerji under whose directions this work was carried out, and for help and encouragement during the investigation.

Literature Cited.

1. Banerji, E. A.—(1931) Cytology of Capsicum (Unpublished M. Sc. Thesis, Cal. Univ.).
2. Belling, J. and Blakeslee, A. F.—(1923) The Reduction division in haploid, diploid, triploid and tetraploid Daturas. Proc. Nat. Acad. Sci. 9 : 106–111.
3. Belling, J. and Blakeslee, A. F.—(1922) The assortment of chromosomes in triploid Daturas. Amer. Nat. 56 : 339–346.
4. Blakeslee, A. F. Belling, J. and Farnham, M. E.—(1923) Inheritance in tetraploid Daturas. Bot. Gaz. 76 : 329–373.
5. Clausen, R. E. and Goodspeed, T. H.—(1925) Interspecific Hybridization in Nicotiana. Genetics. 10 : 278–284.

6. COOPER, D. C.—(1931) Microsporogenesis in Buginvillaea Glabra. Amer. Journ. of Bot. 18 : 337–358.
7. ——————— Macrosporogenesis and the development of the macrogametophyte of Lycopersicon esculentum. Amer. Journ. of Bot. 18 : 739–748.
8. COULTER, J. M. and CHAMBERLAIN, C. J.—(1903) Morphology of Angiosperms. Part II. 1903.
9. DIGBY, L.—(1910) "The Somatic, Pre-meiotic and Meiotic Nuclear divisions of Galtonia Candicans". Ann. of Bot. 24 : 727–757.
10. ——————(1912) The Cytology of Primula kewensis and of other related Primula Hybrids. Ann. of Bot. 26 : 357–388.
11. ——————(1919) On the Archesporial and Meiotic Mitoses of Osmunda. Ann. of Bot. 130 : 135–172.
12. DUGGAR, B. M.—(1899) On the development of the pollen grain and embryo sac in Bignonia venusta. Bull. Torrey Bot. Club. 26 : 80–105.
13. EVANS, A. T.—(1919) Embryo sac and Embryo of Pentstemon Secundiflorus. Bot Gaz. 67 : 427–437.
14. FARMER, J. B. and MOORE, J. E. S.—(1905) On the Meiotic Phase in Animals and Plants. Quart. Journ. Micr. Sc. 48 : 489–557.
15. FERGUSON, M. C.—(1927) A cytological and a genetical study in Petunia I. Bull. Torrey Bot. Club. 59 : 657–664.
16. FIKRY, M. A.—(1930) Phenomena of Heterotypic Divisions in the Pollen Mother cells of a Tetraploid form of Rumex scutatus. Var. Typicus. Journ. Roy. Micr. Soc. 50 : 387–419.
17. GATES, R. R. and REES, E. M.—(1921) A Cytological study of Pollen Development in Lactuca. Ann. of Bot. 35 : 364–398.
18. GATES, R. R. and LATTER, J.—(1927) Observations on the Pollen Development of Two Species of Lathraea. Journ. Roy. Micr. Soc. 47 : 209–225.
19. GUIGNARD, L.—(1882) Recherches sur le Sac Embryonuaire des Phanérogames Angiospermes. Ann. Sci. Nat. Bot. 13 : 136–199.
20. JORGENSEN, C. A.—(1928) The experimental formation of heteroploid plants in the genus Solanum. Journ. of Genetics. 9 : 133–211.

21. KOJIMA, H.—(1925) On the Meiosis and Chromosome number in Different races of Solanum melongena. Bot. Mag. Tokyo. 39: 119–123.

22. KOSTOFF, D.—(1926) Formation of pollen by some varieties of Capsicum annum. Ann. Univ. Sofia. Fach. Agric. 4: 101–124.

23. LESLEY, M. M.—(1926) "Maturation in diploid and triploid tomatoes" Genetics 11: 267–279.

24. MAHESHWARI, P.—(1929) Contributions to the morphology of Boerhaavia diffusa (I). Journ. Ind. Bot. Soc. 8: 219–234.

25. MCCLUNG, C. E.—(1929) Handbook of Microscopical Technique. Hoeber. New York.

26. NANETTI, A.—(1912) Sulle problli cause della partenocarpia del Solanum muricatum Ait. Nuov. Giorn. Bot. Ital. N. S. 19: 91–111. (Abstract from Schnarf, K—Embryologie der Angiospermen).

27. PALM, B.—(1922) Zaadvorming en zaadsteriliteit in Deli-Tabak. Bull. Van het Déli Proefstation to Medan-Sumatra. 16. (Abstract from Schnarf, K—Embryologie der Angiospermen).

28. RUTGERS, F. L.—(1923) The female gametophyte of Angiosperms Ann. Jard. Bot. Buitenzorg. 33: 1–66.

29. SANTOS, J. K.—(1928) A Cytological Study of Cocos Nucifera. Philipin. Journ. of Sci. 37: 417–437.

30. SCHERTZ, F. M.—(1919) Early Development of Floral Organs and Embryonic Structures of Scrophularia Marylandica. Bot. Gaz. 68: 441–450.

31. SCHNARF, K.—(1928) Embryologie der Angiospermen.

32. SCHURHOFF, P. N.—(1926) Die Zytologie der Bluttenpflanzen.

33. SOUEGES, R,—(1907) Development et Structure du Tegument Seminal Chez les solanacees. Ann. Sci. Nat. Bot. 6: 1–124.

34. SVENSON, H. G.—(1926) Zytologische-embryologische Solanazeen-studien. I Uber die Samenentwicklung von Hyoscyamus niger L. Svensk. Bot. Tidskr. 20: 420–434.

35. WINIWARTER, VON H.—(1900) Reserches sur l'ovogénèse et l'organogénese de l'ovaire des mammifères. Arch. d. Biol. 17: 33–199.

36. WINKLER, H.—(1909) Uber die Nachkommenschaft der Solanum-Pfropfbastarde und die Chromosomenzahlen ihrer Keimzellen. Zeitschr. F. Bot. 2 : 1–39.

37. ———(1916) Uber die experimentelle Erzeugung von Pflanzen-mit abweichendem Chromosomenzahlen Zeits. F. Bot. 8 : 417.

38. YOUNG, W. J.—(1922) Potato ovules with two embryo sacs. Amer. Journ. of Bot. 9 : 213–214.

39. ———(1923) The formation and degeneration of germ cells in the potato. Amer. Journ. of Bot. 10 : 325–335.

Explanation of Plates.

All the figures were drawn with a camera lucida. The magnifications and the combinations of lenses used are as follows:—

Figs. 1–3 and 21 were drawn with Leitz Oc. 10 and Obj. 5. Magnification × 650.

Figs. 15–17 were drawn with the same Oc. and Obj. 7. Magnification × 950.

Figs. 4–14 and 18–20 were also drawn with Oc. 10 and Leitz 1 12-inch. imm. N. A. 1.32. Magnification × 1100.

Figs. 24–44, 46, 53–55 and 57 were drawn under a 1/12-inch. imm. Leitz N. A. 1.32, with Comp. Oc. 12. Magnification × 3000.

Figs. 22–23, 45, 47–52 and 56 were drawn under a 1 12-inch. imm. Leitz N. A. 1.32 with Comp. Oc. 18. Magnification × 4500.

Plates IV and V reduced to three-fourth and plate VI to half of its original size.

PLATE I.

Fig. 1. Single hypodermal archesporial cell.
Fig. 2. Two hypodermal archesporial cells lying side by side.
Fig. 3. Two hypodermal archesporial cells one above the other, both in meiotic prophase.
Fig. 4. Homotypic division, both in late anaphasic stage.
Fig. 5. Later stage, four nuclei are in process of reconstruction, and are separated by walls. One of the cells from the chalazal end developing as an archesporial cell.
Fig. 6. A linear tetrad of four megaspores.
Fig. 7. The three other megaspores excepting the chalazal one degenerating.
Fig. 8. Three disintegrating megaspores forming a cap over the developing chalazal one.

PLATE II.

Figs. 9, 10. Showing respectively the second and the third megaspores from the micropylar end degenerating first.

Fig. 11. Both the chalazal megaspore and the next one just above it developing equally vigorously.

Fig. 12. The chalazal megaspore dividing.

Fig. 13. The binucleate embryo sac.

Fig. 14. The binucleate embryo sac dividing to four-nucleate stage.

Fig. 15. Four-nucleate embryo sac dividing to eight-nucleate stage.

PLATE III.

Fig. 16. The eight-nucleate embryo sac. Synergids and egg not yet differentiated.

Fig. 17. The mature embryo sac. The three antipodals completely disintegrated and the two polar nuclei migrating.

Fig. 18. Showing the egg apparatus.

Fig. 19. Fully developed synergids, with prominent beak and large basal vacuole.

Fig. 20. Stages in the fusion of the polar nuclei forming the primary endosperm nucleus.

Fig. 21. Abnormal development of the female gametophyte, showing a second row of four megaspores at the chalazal end of the nucellar tissue.

PLATE IV.

Fig. 22. Resting stage of the nucleus of the megaspore mother cell.

Fig. 23. Early prophasic stage.

Fig. 24. Prophase, showing the parallelism of the linin threads at places and the beaded appearance of the same. (The nucleus cut transversely).

Figs. 25, 26, 27. Showing the formation of a dense tangle of univalent leptotene threads with occasional fission in their lengths. The nucleolar protrusion and swelling at the point of intersection of the threads is conspicuous.

Fig. 28. Contraction of the spireme just starting.

Figs. 29, 30. Later stages of first contraction.

Fig. 31. Full contraction.

Figs. 32, 33. Opening of the spireme as distinct loops. Marked alveolization is still visible in the lengths of the spireme.

PLATE V.

Figs. 34, 35. Hollow spireme stage. The nucleolus in Fig. 35 is displaced by the knife where the nucleolar protrusion is very conspicuous.

Fig. 36. Same stage showing early pairing of the univalent threads taking place at the bases of the loops.

Fig. 37. Pachynema stage.

Fig. 38. Stage leading to second contraction. The loops are drawn again from the periphery towards the nucleolus.

Fig. 39. A stage just after the second contraction. The nucleolus comes back at the centre and the threads are thrown out again as loops. The bivalent nature of the thread is evident at places.

Fig. 40. Separation of the univalents and marked swelling at the points of union.

Figs. 41, 42. The spireme is segmented into twelve 'U' 'V' '8' shaped bivalent chromosomes.

Fig. 43. The bivalents are markedly condensed, but still scattered throughout the nuclear cavity. They are connected with each other by fine strands.

Fig. 44. Diakinesis stage. The gemini taking a peripheral position. The nucleolus is still present with the characteristic nucleolar protrusion.

PLATE VI.

Fig. 45. The gemini collecting at the centre of the bipolar achromatic figure.

Fig. 46. Heterotypic metaphase stage.

Fig. 47. Polar view of heterotypic metaphase, showing the 12 haploid bivalent chromosomes. Each univalent is bean-shaped.

Fig. 48. Early anaphase stage, showing the attachment between univalents by bonds and the traction fibres.

Fig. 49. Showing different modes of attachment of univalent chromosomes between each other during early anaphase.

Fig. 50. The univalents moving towards the poles of the spindle.

Fig. 51. Polar view of the same. Two plates of twelve univalent chromosomes in different foci. Note the spherical outline of the chromosomes.

Fig. 52a. Side view of the spindle near one pole. (Reconstructed from two consecutive sections).

Fig. 52b. Corresponding section showing polar view of the spindle near the other pole. Note the homotypic split in each univalent chromosome.

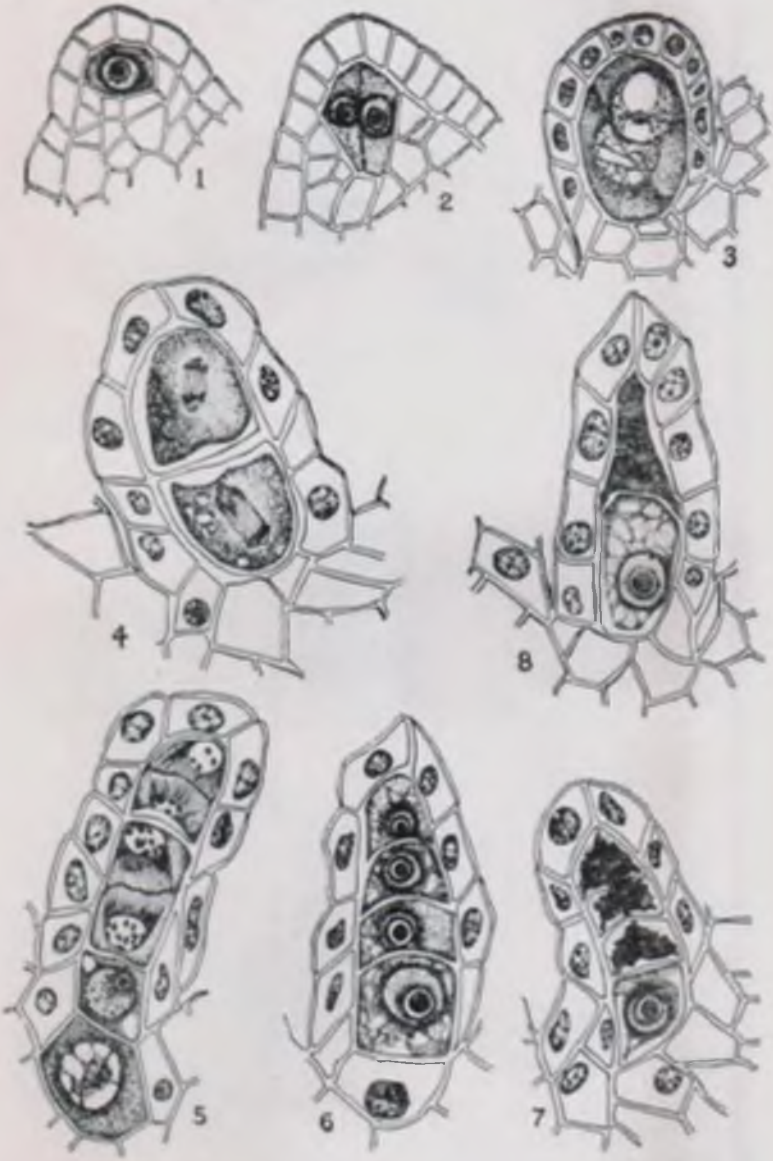
Fig. 53. Late telophase.

Fig. 54. Interkinesis stage.

Fig. 55. Prophase stage, in one of the nuclei in the four-nucleate stage of the embryo sac. Marked alveolization and parallelism in linin threads quite distinct at places.

Fig. 56. Polar view of early metaphase stage in one of the nuclei dividing from four-nucleate to eight-nucleate stage of the embryo sac. Note the twelve, slender, 'V'-shaped chromosomes.

Fig. 57. Chromosome plate showing the somatic number (24) of chromosomes (From a somatic cell of the ovule).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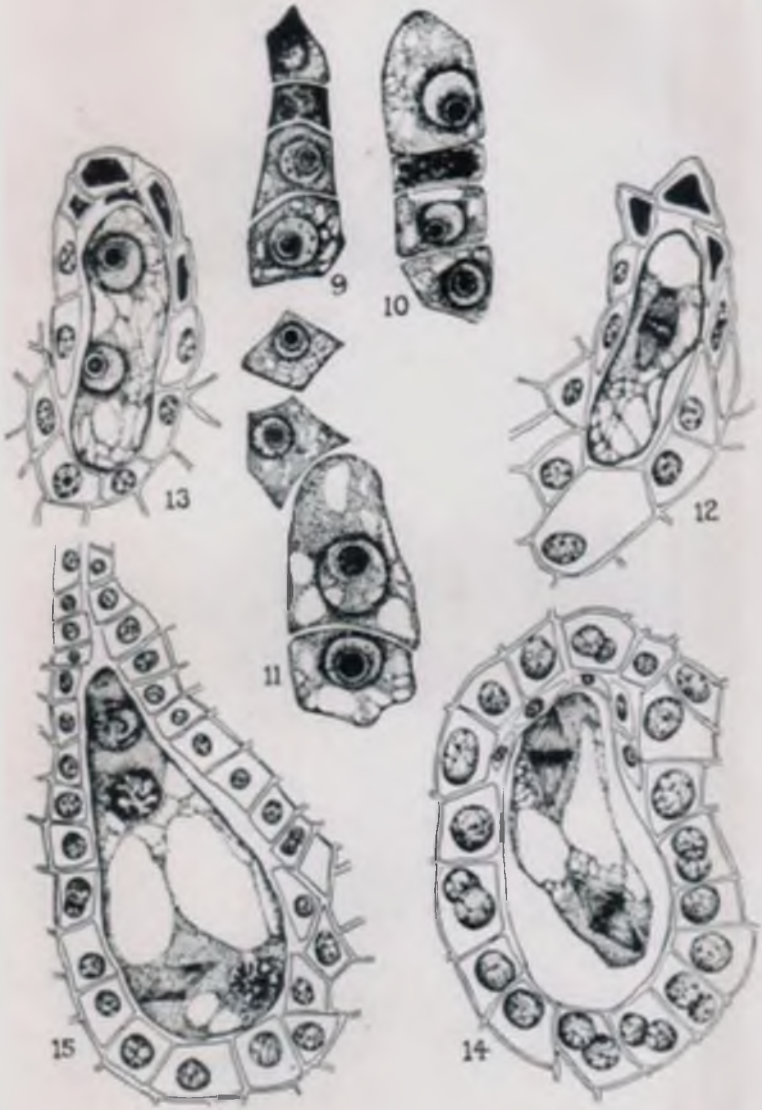
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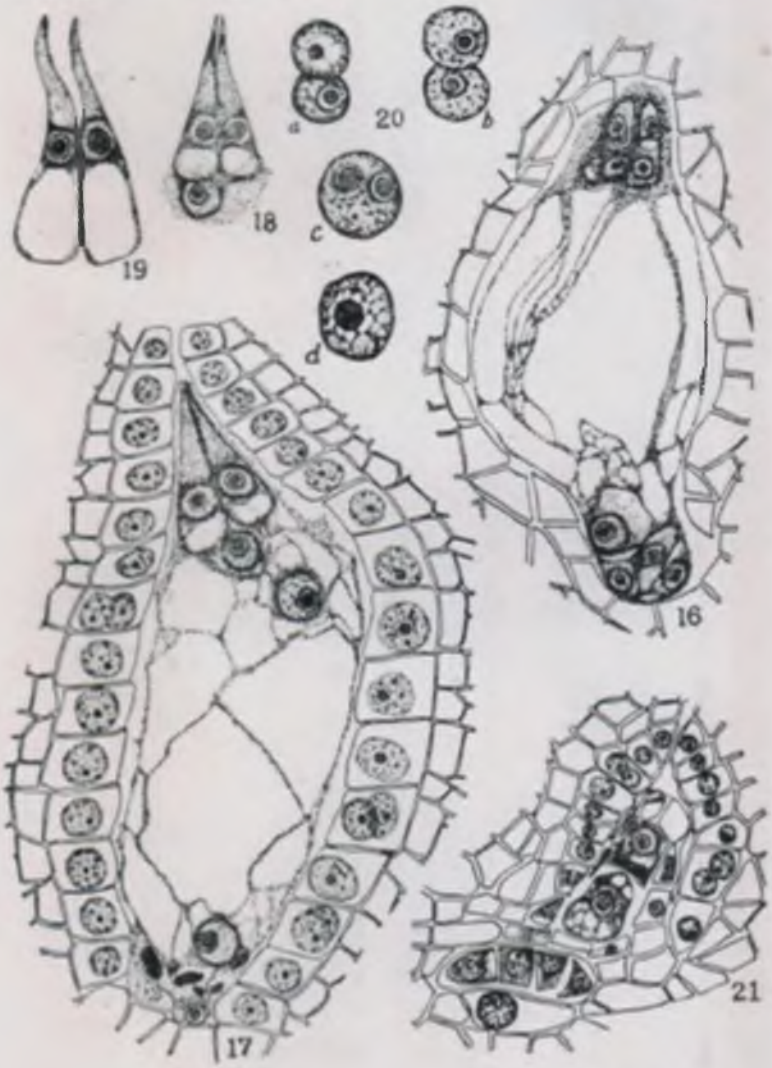
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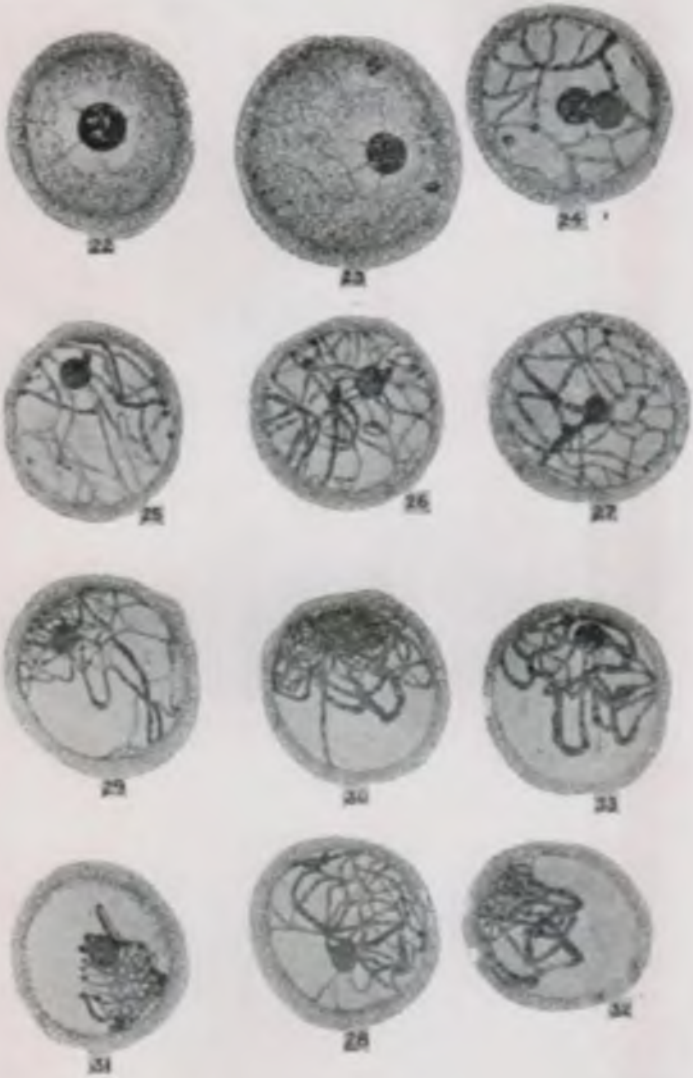

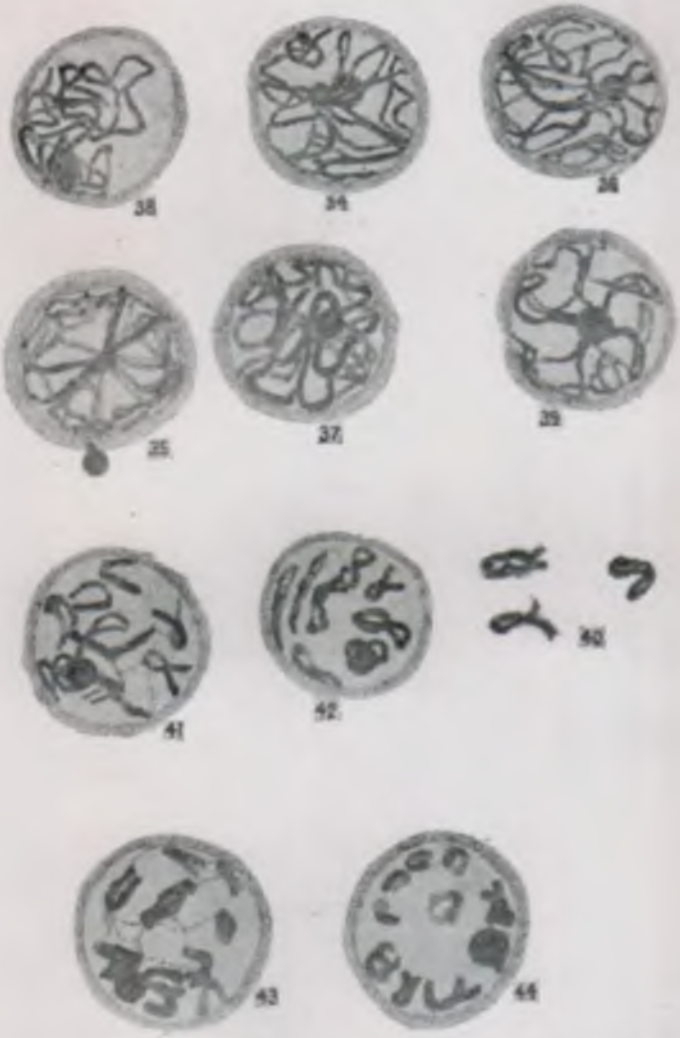
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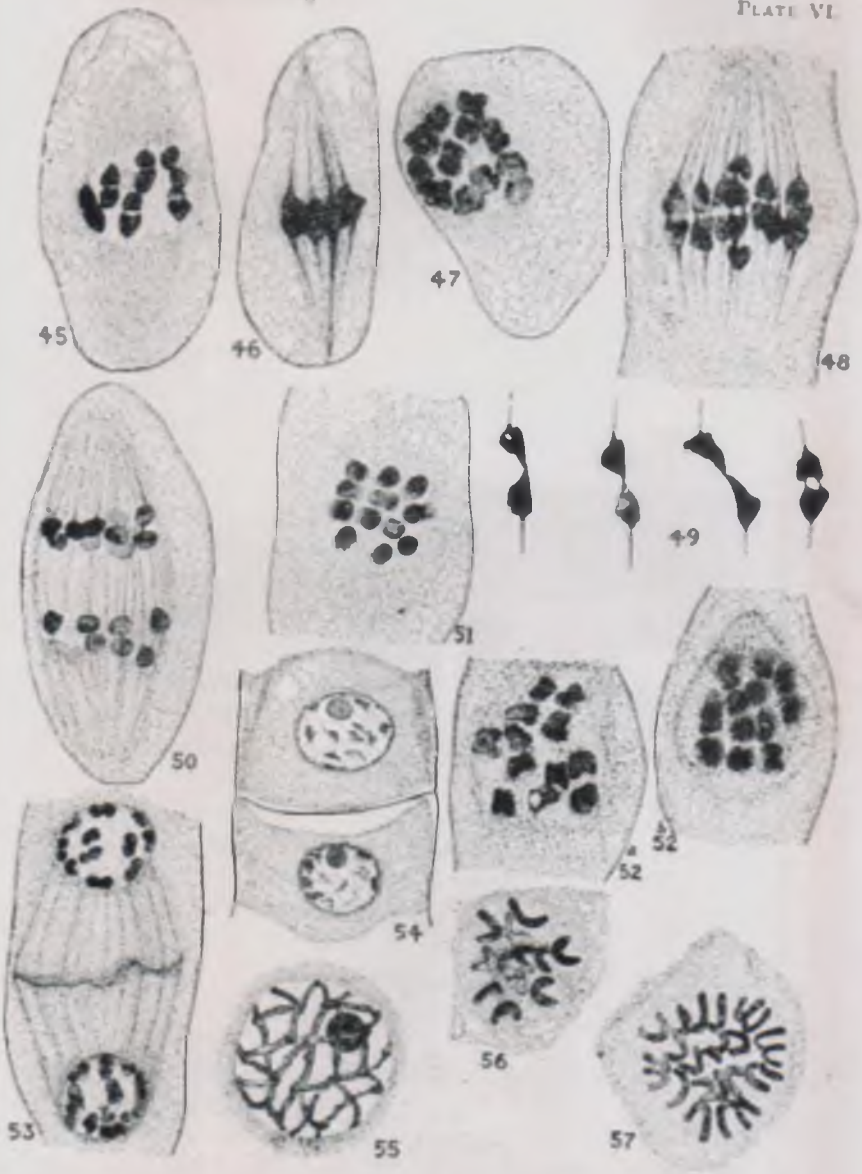
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