CONTRIBUTION TO THE LIFE-HISTORY OF

STELLARIA MEDIA L.

BY PRAKASH CHANDRA JOSHI.

(From the Department of Botany, University of the Panjab, Lahore.)

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1. Introduction.

Stellaria media L. is a member of the sub-family Alsinoideae of the family Caryophyllaceae. It is a plant of very wide distribution, and is most variable in its external characters, several varieties having been recognised. In the Panjab it occurs as a small procumbent and profusely branched annual herb in waste places and also as a weed of cultivation.

Megasporogenesis of Stellaria media has been studied both by Gibbs (1907) and Rocén (1927), but it is seen that the results of the two contradict each other. According to the former, the sub-epidermal primary archesporial cell of the ovule develops directly into a megaspore without undergoing any cell division after the well-known “Lilium-type”. On the other hand, the latter states that the primary archesporial cell definitely cuts off a wall cell to outside, and later a row of four or three megaspores is formed, only the lowest one of which develops into the embryo-sac in a normal manner. Gibbs in his paper has in addition described the complete development of the embryo-sac of Stellaria media and a few stages in the formation of the embryo. Being mostly interested in the nutritive phenomena of the embryo, she did not follow the actual sequence of its cell divisions.*

Rocén's work is quite recent, but as it extends to several families and numerous species of the order Centrospermales, it does not appear to be very intensive in respect of any single species. On the contrary, Gibbs' account, although old, is confined only to the sub-family Alsinoideae and mostly to Stellaria media. It was, therefore, suggested to the writer by the late Prof. S. R. Kashyap to revise the previous work on megasporogenesis of the plant and further to study the development of its pollen-grain, regarding which nothing has been done so far.

* The writer has undertaken to deal with full embryogeny in a separate paper.
2. Material and Method.

The material for the present investigation was collected from Lahore during the winter months of 1933. Flowers were fixed at different hours of the day in various fluids. Allen’s modification of Bouin’s fluid P.F.B.15 (McClung, 1929) gave best results for younger stages and formalin-acetic-alcohol for older ones. After imbedding in paraffin, sections were cut 6 to 10 μ in thickness. Haidenhain’s iron-alum haematoxylin was mostly used for staining. Orange G. in clove oil proved to be quite a good counter-stain with it. A concentrated solution of picric acid was also used for destaining instead of 2% iron-alum solution, and it served the purpose satisfactorily.

3. Investigation.

Microsporogenesis.—The young stamen is early differentiated into a basal filament and a terminal anther. The anther is at first a homogeneous mass of small meristematic cells covered externally by an epidermis. In its outline it is almost circular in a cross section. With continued growth it becomes oval, later bi-lobed and then faintly four-lobed. The primary archesporium is differentiated almost simultaneously with the appearance of the four lobes, and it consists of four bands of hypodermal cells, one in each lobe. Each band is four to six cells long (Fig. 2) and two cells broad (Fig. 1), but at the upper and lower ends it is generally reduced to only a single cell in width. A fairly extensive archesporium has also been reported in *Digera arvensis* (Joshi and Rao, 1934) and *Portulaca oleracea* (Cooper, 1935) from among the members of the Centrospermales so far investigated. The archesporial cells are, as usual, easily distinguishable from the rest of the cells of the anther by their larger size, larger nuclei and more densely staining cytoplasm. The anther lobes become more and more prominent after the differentiation of the primary archesporium.

The primary archesporial cells divide. The first division takes place in a periclinal direction, thus giving rise to a layer of primary wall or parietal cells to the outside and a layer of primary sporogenous cells to the inside (Fig. 3). The primary parietal cells increase in size, and undergo both periclinal and anticlinal divisions to form three to four cell layers of the parietal tissue situated in between the epidermis and the sporogenous cells of the anther (Figs. 4 and 5). The outermost parietal layer later becomes differentiated into an endothecium and the innermost layer into a tapetum. The sporogenous cells take no part in the formation of the latter. The nature of the tapetal cells is described later on.

The division of the sporogenous cells occurs (Fig. 4), and as a result of this two to five (most commonly four) microspore mother-cells are seen
in a cross section of an anther lobe (Fig. 5), the number decreasing towards the two ends. All the cells divide neither simultaneously nor in the same plane. On the whole about twenty microspore mother-cells have been counted in each sporangium. Thus roughly speaking, each primary sporogenous cell undergoes only one mitotic division. As each mother-cell later on produces four microspores, the total output of pollen of a single stamen of *Stellaria media* may, therefore, be estimated to be nearly 320.

The tapetal cells show the usual characters—oblong shape in section, broad, large nuclei, richly protoplasmic contents and a deeply staining capacity. At first the cells are uninucleate but later the nuclei divide. This occurs about the time of diakinesis or a bit earlier. The division is mitotic as has been described by Cooper (1933) in many other plants. The tapetum attains its maximum development soon after the second meiotic division (Fig. 16). Occasionally three or four nuclei have been noticed in a single tapetal cell, and a single nucleus may contain two or three nucleoli. Another feature of the tapetum of *Stellaria media* is the entire lack of vacuoles. The tapetal cells, moreover, always remain at the periphery even when their inner walls become disorganised, and never move inwards to form any kind of periplasm-odium.

The spore mother-cells undergo a fairly long period of rest, and during this interval the size of their cells and nuclei increases. Unlike flowering plants in general, the mother-cells neither round off themselves and separate from each other at this stage, nor do their walls become thickened. They remain packed together as is the case in *Digera* (Joshi and Rao, 1934), *Althaea*, etc. (Coulter and Chamberlain, 1903). Coulter and Chamberlain attribute this latter condition to limitation of space due to tardy disorganisation of tapetum or its failure to disorganise, but this explanation does not hold good for *Stellaria media*. In this plant at this period the anther enlarges considerably in size, and both the tapetum and spore mother-cells fail to keep pace with it. As a result of this (stretch) the walls of the tapetal cells break one after another, and a space arises in between the tapetum and the mass of sporogenous cells. The spore mother-cells, packed together, float in the cavity of the sporangium. They show the first sign of rounding at about the time of diakinesis. The spore mother-cell just before entering the first or the heterotypic meiotic division is polygonal in outline and has a thin cell wall. It is completely filled with cytoplasm which is alveolar in structure and is devoid of any deeply staining matter. In its centre it has one spherical nucleus which has got a fine nuclear membrane. Inside the nucleus there is a single, rather large, spherical nucleolus which stains intense black with haematoxylin (Fig. 6). The nucleolus is located either in the centre or on
Contribution to the Life-History of Stellaria media L.

Note:—All the figures are from *Stellaria media*, and have been drawn with the help of a camera lucida. Magnifications given are only approximate.

Shows various stages of microsporogenesis.

Fig. 1.—Part of t.s. of a young anther, showing two primary archesporial cells in a single anther lobe. × 900.

Fig. 2.—L.s. of a single lobe of a young anther, showing five primary archesporial cells. × 900.

Fig. 3.—L.s. of an anther lobe, showing the division of the primary archesporial cells into primary wall cells and primary sporogenous cells. × 900.
The heterotypic division commences with an increase in the density of the karyolymph, and soon after this the linin threads make their appearance in the form of a net-work at the periphery (Fig. 7). Numerous minute and faintly staining granules later become distinct on the linin, and it resolves into the usual reticulum (Fig. 8). It fills the whole nuclear cavity. After some time the thread begins to retract from the boundary, and contracts towards one side of the cavity into a knot. This contraction lasts for a fairly long time, but throughout this interval the outline of the thread can be made out. There is no sudden enlargement of the nucleus at this stage, it, therefore, does not support Lawson's (1911) view that synizesis results from an all of a sudden growth of the nuclear cavity. The growth is only slow and steady. The nucleolus is never enclosed in the synizetic knot but it lies near it on one side. The two have been seen connected with each other by a fine thread in many cases, which indicates that they are in a very close association with each other. In some cases small, spherical and deeply staining bodies, similar to "nucleolar bodies" recorded by Cleland (1922), Latter (1926), Sethi (1930), etc., have been observed lying at the circumference of the nucleolus. Occasionally these are found connected with the spireme (Fig. 9). During the contraction fragments of dark staining material are seen lying on the contracted thread, and its amount of chromatin increases. In some cases simultaneously with this and in other cases a bit later the staining capacity of the nucleolus decreases (Figs. 5, 10 and 11). The decrease begins from the periphery towards the centre, and ultimately the whole body takes a very light stain. This behaviour suggests very strongly that during synizesis the nucleolus passes out its stainable material to the thread. Gregory, Wager, Cardiff, Sethi and many others (quoted in Sethi, 1930 and
Contribution to the Life-History of Stellaria media L.

Sharp, 1934) hold that the nucleolus serves as a store-house for the bulk of the chromatin material during the resting periods which intervene between successive mitoses. The same seems to be true for Stellaria media. The nucleus after the completion of the synizetic contraction enters the open spireme stage in which the thread seems to be continuous, and is comparatively more dense and thicker (Fig. 11). When just coming out of the contraction the thread shows concentration of chromatin at certain places to form numerous deeply staining bodies separated by lighter portions of the thread. Uptil this the thread seems to be univalent in nature and no split is perceptible at any place. No second contraction takes place. The thread is thrown into loops and this is followed by diakinesis stage. The exact changes which lead to the pairing of chromosomes could not be made out due to their rather large number and very minute size. Soon after the diakinesis the nuclear membrane and usually the nucleolus also disappear. Most of the further stages in meiosis were missing in the preparations examined. The number of bivalent chromosomes could be counted as twenty at the metaphase plate of the heterotypic division when cut across (Fig. 12). This confirms the haploid number as given by Rocén for the species. After the first telophase, the two groups of chromosomes on the two opposite poles of the spindle form two daughter nuclei with definite surrounding membranes (Fig. 13). No evanescent cell plate at the equator of the spindle could be made out. In one case two chromosomes were seen to lag behind on the spindle fibres while the daughter nuclei had been formed. In a few cases the primary nucleolus was seen to persist upto the late telophase (Fig. 13), which is rather curious. No wall is formed between the two daughter nuclei which again undergo the second meiotic division. The spindles of this division appear simultaneously and are mostly situated at right angles to each other (Fig. 14), but a parallel arrangement of them (Fig. 15) is also not rare. Soon after the four grand-daughter nuclei have been formed at the poles of the two primary spindles, there appear secondary spindles of achromatic fibrils, and these connect the nuclei in all possible directions (Fig. 16). These secondary spindles persist for a considerable time. Cytokinesis takes place by cleavage of the cytoplasm (Fig. 17) in a manner as described by Farr (1916). No vacuoles similar to those reported by Castetter (1925) in Melilotus were seen.

The pollen-grain.—The pollen-grains in the tetrad are arranged both tetrahedrally and bilaterally (Figs. 18–20). They get free from each other by the dissolution of the wall of the mother-cell. The degeneration of one, two, three or even four pollen-grains of a tetrad, while still inside the mother-cell wall, is quite common. The degenerated pollen-grains stain dark black.
The free microspores in the anther-halves round up and increase in size, and, as usual, a thick exine and a thin intine get differentiated, and the cytoplasm becomes vacuolated (Figs. 21-23). The nucleus divides into a large tube nucleus and a small generative nucleus (Figs. 23-24). No cell wall appears in between them, and the latter was never seen to organise itself into a cell. The further division of the generative nucleus of the pollen-grain inside the anther was seen to take place only in a few cases. It forms two similar male nuclei with definite nucleoli (Fig. 25). Perhaps the pollen-grains at the time of shedding are both binucleate and trinucleate.

The ripe pollen-grains vary much in size, measuring from 18μ to 30μ in diameter. Most of the large grains get degenerated (Figs. 27-29), their cytoplasm stains dark, and the nuclei undergo fragmentation. In some cases the nuclei were seen to divide in a mitotic manner even after the signs of degeneration had appeared (Figs. 27-28), and in one instance nearly twenty chromosomes could be counted very clearly. One normal pollen-grain was found to be four nucleate (Fig. 26). The extra nucleus was very small, and it occupied a position just near the pair of two male nuclei towards the periphery. In the Centrospermales a "prothallial cell" has been recently reported by Billings (1934) in *Atriplex hymenelytra*, but there is no scope for such an interpretation in the present case. According to Coulter and Chamberlain (1903), any cell (or nucleus) may be induced to divide under favourable conditions. In *Lilium, Asclepias*, etc., tube nucleus fragments, and forms additional nuclei. Schnarf (1929) thinks that all such instances of excessive nuclei in the male gametophyte, irregularities in meiosis and defective pollen development in angiosperms are mostly due to hybridisation.

The tapetum and all the wall layers of the anther except the outer epidermis and the endothecium begin to disintegrate after the formation of the pollen tetrads, and gradually disappear. The walls of the endothecial cells get thickened (Fig. 23), and in a mature anther these cells develop the characteristic fibrous bands.

**Megasporogenesis.**—The rudiments of the campylotropous ovules appear as small conical protuberances from the central placenta of the gynæceum in a basipetal succession as described by Gibbs. At first each of them consists of only a few homogeneous meristematic cells, but later the cells grow and multiply. The primary archesporium differentiates very early before the initials of the integuments are laid. It is hypodermal in origin and consists of four to six cells at the top of the nucellus. These are distinguishable, as usual by their large size, large nuclei and deeply staining
Shows various stages of microsporogenesis and development of the pollen-grain.

Fig. 16.—Part of l.s. of the anther, showing fully developed two-nucleate tapetum and the microspore mother-cells in telophase of the second meiotic division. × 900.

Fig. 17.—Shows cytokinesis by furrowing. × 1350.

Figs. 18–19.—Show the different arrangement of microspores formed from the same mother-cell. × 900.

Fig. 20.—Shows degeneration of two young pollen-grains in the tetrad. × 900.

Fig. 21.—Pollen-grain with a thin undifferentiated wall. × 900.
Fig. 22.—Pollen-grain, showing differentiation of the wall into intine and exine. × 900.

Fig. 23.—Part of l.s. of anther, showing thickening of the endothecial layer and complete differentiation of the wall of the pollen-grains, which are both 1- and 2-nucleate. × 900.

Fig. 24.—Pollen-grain with its nucleus in telophase of the first division. × 900.

Fig. 25.—Pollen-grain with three nuclei, the tube nucleus and the two male nuclei. × 900.

Fig. 26.—A 4-nucleate pollen-grain. × 900.

Figs. 27–29.—Degeneration of pollen-grains; two show mitotic division of nuclei even after the signs of degeneration have appeared. × 900.

cytoplasm. Fig. 30 shows the presence of three archesporial cells in a single longitudinal section of the ovule. The writer's observations are against the statement of Gibbs that there is only a single archesporial cell in the ovule of the species. *Silene, Melandrium, Agrostemma, Dianthus, Lychnis* and *Scleranthus* are other genera of the Caryophyllaceae in which more than one archesporial cells have been noted (Schnarf, 1931).

All the primary archesporial cells of an ovule do not develop further in the same manner. Generally, only one or, less frequently, two located in the centre function; the rest lose their identity and become indistinguishable from the nucellar cells. The functional archesporial cell increases in size, and then divides transversely or obliquely to form the primary wall or parietal cell and the primary sporogenous cell (Figs. 31–33). The sporogenous cell, as usual, is itself a megaspore mother-cell. In very rare cases an archesporial cell enlarges and becomes a megaspore mother-cell without previously cutting off a parietal cell to the outside. This is apparent from Fig. 34 which shows a comparatively large cell with its nucleus in the synizesis stage of the first meiotic division and no wall cell on its outside. The non-formation of a wall cell has been described as a general condition by Gibbs in the Alsinoideae and by Souèges (1926) in *Sagina procumbens*. Rocèn, on the other hand, has strongly contradicted these previous observations. Out of the numerous species studied by him, he could find only in case of *Gypsophila perfoliata* that sometimes the archesporial cell prepares for the heterotypic nuclear division without first separating off a wall cell.

The further development of the megaspore mother-cell is variable, and the results of the present investigation are at variance with those of Gibbs and Rocèn. A row of four or three megaspores, formed as a result of the usual two successive divisions of the mother-cell, was never seen. Only in a few cases the mother-cell had divided to form two superposed daughter cells with a transverse or an oblique wall in between (Figs. 35, 36). From a large number of slides examined nothing could be made out of the further behaviour of these two components of the dyad. Perhaps they do not
Shows various stages of megasporogenesis. × 900.

Fig. 30.—Apex of the nucellus, showing three cells of the primary archesporium.

Fig. 31.—One functional archesporial cell has divided to form a primary wall and a primary sporogenous cell (Megaspore mother-cell).

Fig. 32.—Two archesporial cells have divided to form a primary wall cell and a primary sporogenous cell.

Fig. 33.—Ovule showing the enlargement and elongation of the megaspore mother-cell.

Fig. 34.—The primary archesporial cell has developed into an embryo-sac cell without undergoing any division; nucleus in synizesis.

Figs. 35-36.—Dyad of two megaspores with an oblique or transverse wall in between.

Fig. 37.—The embryo-sac with its primary nucleus in telophase.
undergo any further division, but one of them enlarges to form directly the embryo-sac, the other merging into the nucellar cells in a manner similar to the non-functional archesporial cells. This probable suggestion does not preclude the possibility that the two megaspores may give rise to three or four megaspores which is held by Rocén as a constant condition for the species. In the majority of the ovules it is seen that the megaspore mother-cell does not divide at all, but itself functions as the initial of the embryo-sac. Figs. 34 and 37 show that it increases in size, and elongates rapidly as is usual with embryo-sacs in other plants. The primary nucleus divides into two. The division is not followed by wall formation and the result is a two-nucleate embryo-sac.

The above account shows that Gibbs and Rocén both are partly justified in their statements concerning megaspore formation in *Stellaria media*. Neither of them recorded any variation in the process or thought of its possibility. That is why contradictory statements have appeared, and this may be due to an examination of a small amount of material by each. On the other hand, externally *Stellaria media* is one of the most variable plants, and it may also be varying internally in this respect, in one variety one kind of megaspore formation may be more frequent. Rocén (1927) himself observed in *Mirabilis jalapa* and *Oxybaphus nyctagineus*, belonging to Nyctaginaceae, rows of four, three and two megaspores and also the mother-cell functioning directly as a megaspore. It seems that this condition may be true for many more plants of the Centrospermales besides these examples.

The Embryo-sac.—One nucleus of the two-nucleate embryo-sac moves towards its micropylar end and the other towards the chalazal one. The embryo-sac continues to increase in size, especially lengthwise, and in the meanwhile vacuoles appear in the cytoplasm, and one in the middle portion of the embryo-sac becomes quite large (Fig. 38). The two nuclei at the two ends divide twice to produce the final eight nuclei of the adult embryo-sac (Figs. 38–40).

The fully-formed embryo-sac of *Stellaria media* is of the usual organisation, consisting of an egg-apparatus, three antipodals and two polar nuclei (Figs. 40, 41). Gibbs has said very little about these various components, and the diagrams given are too small to be fully illustrative, a brief account of them, therefore, seems desirable.

The egg-apparatus at the micropylar end consists of three elongated pear-shaped cells, the egg being somewhat larger than the two synergids. In a frontal view the synergids lie side by side almost touching by their lateral walls, and they cover a major portion of the egg, only its rounded distal end
Shows development of the embryo-sac.

Fig. 38.—A two-nucleate embryo-sac; some of the cells on the periphery of the embryo-sac have been disintegrated by its enlargement. × 900.

Fig. 39.—A 4-nucleate embryo-sac. × 900.

Fig. 40.—A young fully-formed embryo-sac (Re-constructed). × 900.

Fig. 41.—Embryo-sac at a later stage; the antipodals are in a process of degeneration (Re-constructed). × 450.

Fig. 42.—Embryo-sac after fertilisation, showing the remains of a pollen tube on one side of the undivided oospore, a few endosperm nuclei and the last remains of antipodals; the synergids have disintegrated altogether (Re-constructed). × 450.

being visible. During early stages of development the three cells of the egg-apparatus are seen completely full of cytoplasm, but, as usual, with continued growth they become vacuolated. In each synergid a large vacuole appears in the basal region of the cell, and the nucleus is embedded in
cytoplasm just above this vacuole. The synergids are devoid of every kind of
filiform apparatus or hooks. In case of the egg the vacuole appears in the
apical region, and its nucleus lies in the broad basal part of the cell surrounded
by an extremely dense cytoplasm. The mature egg very much exceeds a
synergid in the size of its cell and the nucleus.

The three antipodal cells at the chalazal end of the embryo-sac are more
or less triangular in shape. Their arrangement is variable; generally, they
lie close to one another at the same height, but in some cases one lies just at
the bottom of the embryo-sac and the other two lie above it in close proximity.
Each antipodal is at first surrounded externally by a membrane like the cells
of the egg-apparatus, but later it becomes covered by a definite cell wall.
The antipodals of *Stellaria media* are ephemeral in nature like those of other
caryophyllaceae. They begin to degenerate early, and disappear altogether
about the time of the first division of the fertilised egg.

The two polar nuclei, as described by Gibbs, fuse at the micropylar end
to form a very large secondary nucleus which comes to lie very close to
the egg.

Gibbs thinks that after fertilisation the first division of the oospore
always precedes the first division of the endosperm nucleus, but the writer
has found the reverse to be a constant condition in all the preparations
examined for the purpose (Fig. 42). At the time of the first segmentation
of the egg there are always found four to eight free endosperm nuclei in the
embryo-sac. The division of the endosperm nuclei is mitotic, but sometimes
figures similar to those of the amitotic one are also seen.

4. Summary.

1. The primary archesporium in each sporangium of the anther of
*Stellaria media* consists of several cells.

2. Microsporogenesis is almost typical. The mother-cells do not
round off and separate from one another prior to division. During synizesis
staining material of the nucleolus appears to pass out to the spireme which
gains in its chromatin content. Cytokinesis takes place by furrowing.

3. The tapetum is formed from parietal tissue alone. At about the
time of diakinesis the nuclei of the tapetal cells divide mitotically, and the
tapetal cells become binucleate.

4. The pollen output of each anther appears to be about 320. The
pollen grains are perhaps shed in both 2- and 3-nucleate condition. The
generative nucleus has not been seen to organise a cell.
5. The primary archesporium in the ovule consists of 4–6 cells, one of which functions ultimately.

6. Usually the functional archesporial cell divides to form a primary wall cell on the outside and a primary sporogenous cell (megaspore mother-cell) on the inside, but in rare cases no wall cell is cut off.

7. The megasporogenesis is variable. In no case a linear row of 4 or 3 megaspores was seen, but in a few cases a dyad formed from a mother-cell was seen. In majority of cases a mother-cell becomes itself the embryo-sac cell.

8. The fully-formed embryo-sac is an 8-nucleate structure of the usual organisation.

9. The primary endosperm nucleus always divides before the fertilised egg.

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