A CYTOLOGICAL STUDY OF SCAEVOLA LOBELIA LINN.

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Introduction

The literature in the family Goodeniaceae as reviewed by Schnarf (1929, 1931) reveals that only two members, Scaevola Koenigii and Dampiera stricta have been investigated. The work of Billings (1901) on Scaevola treats of the embryology of the plant, while that on Dampiera by Brough (1927) relates to the development of pollen and the formation of the embryo sac. A detailed cytological investigation does not seem to have been undertaken in the family so far.

The material for the present study, in which a detailed examination of the important stages in the meiotic cycle of the microspore mother cells is made, was collected during October 1937 in the island of Krusadai, near Pamban, South India. There are only a few plants on the sandy shore of the island, well above the high tide mark and about a furlong from the Marine Biological Laboratory. The plants are small in stature and thick-set with large succulent leaves. The flowers are conspicuous with a bright yellow and fleshy corolla, which is strongly zygomorphic.

The material was killed in Bouin’s fluid and subjected to the usual course of treatment. Sections were cut varying in thickness from 8 to 10 μ and stained in Heidenhain’s iron alum haematoxylin.

Structure of the Anther

The wall of the anther shows an epidermis, an endothecium, a single middle layer and the tapetum (Fig. 2). All the layers within the epidermis are formed, as usual, from a primary parietal layer, which in itself is derived from the hypodermal archesporium at an early stage. After the separation of the wall layers, the sporogenous cells appear in the form of four greatly elongated cylindrical masses at the four corners of the anther (Fig. 1). The sporogenous cells are sharply separated off from the tapetal cells by the presence of dense cytoplasm and large conspicuous nuclei. The tapetal cells are uninucleate to begin with and contain one or more vacuoles. As the nuclei of the microspore mother cells approach the diplotene stage in meiosis,
the nucleus of each tapetal cell divides once mitotically to form two nuclei (Fig. 3a and b). These nuclei divide again for the second time a little later (Fig. 3c), so that the tapetal cells become quadri-nucleate. Sometimes, however, only three nuclei, one large and two small, may also be formed if the disposition of the spindles of the second division is such that the poles are near each other and the result is the inclusion of the daughter chromosome groups within a common nuclear membrane. The tapetal cells finally become uninucleate again by a fusion of all the free nuclei just prior to the decline in the tapetal activity (Fig. 3d and e).

In addition to the normally formed tapetal cells derived from the primary parietal layer described above, some of the sporogenous cells also seem to take part in tapetal function to some extent. Such sporogenous cells are found towards the inside of the sporogenous groups, namely, next the connective tissue of the anther (Fig. 2). These cells are clearly seen to be arrested sporogenous cells to serve a tapetal function. They are not only continuous with the regular sporogenous cells but also have large and conspicuous nuclei, which are practically in the same stage of development as those in the sporogenous cells.

Meiosis in the Microspore Mother Cells

The resting nucleus of the microspore mother cell shows a number of large and darkly staining chromatin masses, which are connected to one another by fine and delicate threads (Fig. 4). With the first sign of meiosis, the chromatin masses become elongated and resolve themselves into a number of threads of different lengths (Fig. 5). The delicate net-like connections are still visible, but become gradually lost as the leptotene stage is reached. The leptotene threads are uniformly distributed inside the nuclear membrane without showing any definite orientation. The threads appear to be beaded with clear diamond-shaped areas in certain portions (Fig. 6).

The leptotene threads next show evidences of coming together in pairs for synapsis, which marks the zygotene. The pairing of the threads seems to commence anywhere along the length of the threads, but is invariably clearer at the ends (Fig. 7). A distinctly double nature marking the chromonemata is seen in particular regions of the threads which have not as yet synapsed. With the completion of zygotene pairing, the threads become uniformly thick and intensely staining to give rise to the typical pachytene stage (Fig. 8). The four-partite nature of these threads becomes obscure, and a “bivalent” nature only, and that in certain portions alone, can now be seen. Further, the pachytene stage is characterised by a definite orientation of the threads with reference to the nucleolus, though intimate connections between the two are at no time evident.
Fig. 1—Outline of transverse section of anther. \( \times 80 \). Fig. 2—Part of anther to show wall layers, tapetum and the microspore mother cells. Note that the mother cells towards the inside appear tapetum-like. \( \times 800 \). Fig. 3 a, b, c, d and e—Tapetal cells at different periods of their activity (explanation in text). a, b and c \( \times 1800 \); d and e \( \times 900 \). Fig. 4—A microspore mother cell with its nucleus in resting condition. \( \times 2700 \). Fig. 5—Resolution of the network of the nucleus to form the threads. \( \times 2700 \). Fig. 6—Leptotene showing the coiled chromonemata forming diamond-shaped areas. \( \times 2700 \). Fig. 7—Early zygotene; synapsis is taking place in certain portions. \( \times 2700 \). Fig. 8—Pachytene. Note orientation of threads and the "bivalent" nature. \( \times 2700 \). Fig. 9—Early diplotene. \( \times 2700 \). Figs. 10 and 11—Mid-diplotene nuclei showing the full haploid set. \( \times 2700 \).
The emergence of the threads from the pachytene stage next becomes manifest by the falling apart of parts of the synapsed threads (Fig. 9) except at regions where the chiasmata are established. It is not possible to determine exactly where the chiasmata are first established. The formation of the chiasmata in all the pairs gives rise to the diplotene stage. Here again a four-partite nature becomes once more evident in some regions.

The number and nature of the chiasmata in the diplotene pairs depend largely on the relative lengths of the pairs themselves (Figs. 10 and 11). Some of the pairs, usually four or five and which are slightly longer than the rest, have three chiasmata each, of which one alone is terminal and the others are interstitial in some long pairs, while all the three chiasmata are interstitial in the other long pairs. The short diplotene pairs have only two chiasmata, one terminal and the other interstitial. A single short pair, however, seems to have a single terminal chiasma from the earliest clearly recognizable condition. This pair is evidently the first to show a separation of its components at the first anaphase (Fig. 17).

The total number of chiasmata formed in all the pairs in a mid-diplotene nucleus is found, from an examination of a number of nuclei, to be approximately twenty, of which nearly 50% are terminal. In slightly earlier stages of diplotene, the proportion of terminal chiasmata is less, namely, about 33%. In late diplotene the total number of chiasmata is reduced to the minimum of fifteen which are completely and regularly terminalized.

Structure of the Chromosomes

The late diplotene pairs reveal that each homologue is composed of two strands which are coiled in opposite directions (Fig. 14, a, b and c). Ordinarily, the two-coiled strands appear like a number of darkly stained cross bars in a less darkly stained matrix. The chromosomes, therefore, have a wavy outline. When, however, the chromosome pairs are examined more critically the coiled nature of the strands composing the homologues becomes evident, especially in some of the pairs which have been a little extra destained or have become pale on account of time. Straining a little further, it is possible to make out in such pairs that the coils possess points of reversal usually situated on either side of the chiasmata. Since, however, the chromosomes are not large it is not possible to state definitely whether changes in the direction of coiling are restricted or are found at random in the homologues. The coiled nature becomes obscure in the first metaphase chromosomes, as these are very small and appear intensely homogeneous.

There is usually a single nucleolus in the nucleus of the microspore mother cell. This shows in the earlier stages of prophase a number of clear spaces, which become less evident later and finally completely disappear from view
with increased affinity of the nucleolus to the stain. With progressive prophasic transformations of the nucleus, a corresponding reduction in the size of the nucleolus is seen. When the nucleolus is seen for the last time before completely and suddenly disappearing at late diakinesis, it reaches its minimum size and no trace of it is left when the chromosome pairs are arranging themselves on the equator of the first metaphase spindle. It does not reappear in the interkinetic nuclei at the conclusion of the first division, but becomes evident again only in the nuclei of the microspores after the second division is over.

The haploid number of chromosomes was found to be eight in a number of first metaphase plates (Fig. 16). This number also corresponds to the number of pachytene threads and the number of diplotene pairs examined in favourable preparations. The counting of the chromosome number was also checked in the first anaphase and second metaphase stages for purposes of confirmation.

After the chromosomes have taken their positions on the equator of the first metaphase spindle (Fig. 15), the anaphase separation begins (Fig. 17). The homologues of one pair appear to move away from each other earlier than those of the other seven pairs. This pair showing the early separation is evidently the one characterized by the possession of a single chiasma in the diplotene stages. After the separation of the homologues of the other pairs is also effected and on the daughter chromosomes reaching the poles the interkinetic nuclei are formed (Fig. 18). These nuclei do not seem to undergo any period of rest, but become ready immediately for the second division which soon follows (Fig. 19).

During the second division, the two spindles lie at right angles to each other (Fig. 19) so that the resulting nuclei are arranged in a tetrahedral manner (Fig. 20). A dense zone of cytoplasm occupies the region of the mother cell between the two spindles (Fig. 19); this zone marks the position of the first division spindle.

After the second division is over, the mother cells form quartets of nuclei connected together by spindle fibres (Fig. 20). These spindles do not take part in the separation of the microspores, which is effected by the formation of advancing peripheral furrows. The separation of the microspores is simultaneous as in a number of cases recorded in literature (Schürhoff, 1926; Schnarf, 1929, 1931). The young microspores are free inside the original mother cell wall (Fig. 21). The latter undergoes gradual dissolution and the microspores are set free in the anther locule as distinct pollen grains.

The pollen grains are spherical in outline and possess a thick exine. The intine is thin and bulges out at the regions of the germ pores which are
three in number. When mature, each pollen grain has a tube nucleus and a generative cell lying towards one side (Fig. 23). The plasma sheath of the latter becomes obscure a little later when the pollen grain is actually liberated from the anther locule (Fig. 24). Brough (1927) states that the pollen grains of *Dampiera* are also two nucleate at the shedding stage.
The wall of the mature anther consists of the same original layers. The endothecium develops fibrillar thickenings. The tapetum is completely disorganized and the single middle layer of the wall of the anther gets usually crushed between the endothecium and the developing pollen grains.

**Discussion**

While convincing, evidence has accumulated in an overwhelming majority of the angiosperms for pointing out the derivation of the anther tapetum from the primary parietal layer, instances are not entirely wanting to indicate the formation of tapetum from the sporogenous cells. Coulter (1898), from a study of a number of species of *Ranunculus*, states that the tapetum is sometimes derived from the sporogenous tissue and sometimes from the primary parietal layer. Singh (1936), in a recent study of *Ranunculus sceleratus* regards that Coulter was perhaps led to conclude that the tapetum is "occasionally derived by the sterilization of the sporogenous cells" on account of a great similarity between the tapetal and the sporogenous cells in their contents and staining reactions. He further remarks: "During recent years the tapetum is shown to have a parietal origin in so many angiosperms that any claim of a different origin will have to be strongly supported".

Joshi and Venkateswaralu (1936), in their studies in the *Lythracea*, state that the "tapetum seems to be derived largely from the outer sporogenous cells". In support of this the authors present two figures (cf. 75 and 76). They do not, however, clearly state whether all or only a part of the tapetum is formed thus and also, whether any process of conversion or sterilization of sporogenous cells takes place.

In *Scaevola Lobelia*, the tapetum is, however, normally formed from the parietal tissue, but in addition, a further contribution to tapetal function is made by some of the sporogenous cells, those lying on the inside next the connective tissue. These, as already noted, lose their characteristic features and become tapetum-like both in appearance, as well as in function. These cells may therefore be regarded as arrested sporogenous cells which become subjected to a process of sterilization at some time during the early prophase. They thus constitute a secondary tapetum and the statement of Coulter (1898) that the tapetum may occasionally be derived by sterilization may not be wholly wrong.

In *Zostera*, according to Rosenberg (1901), the tapetum can be traced back to the divisions of the greatly elongated sporogenous cells on both the inner and the outer sides of the anther locule. Wylie (1904) states that in *Elodea* "the divisions of sporogenous cells on the axial side seem to be of common occurrence and there is probably a regular contribution to the
tapetum from the sporogenous cells in that region. In addition there may be a contribution to the diffuse tapetum on any side by the sacrificing of potential spore mother cells to the nutritive function.”

Gates and Rees (1921) find that in *Lactuca* a “peculiar feature which is associated with the loose arrangement of the tapetal cells is the fact that a variety of transitional stages occur between tapetal cells and the pollen mother cells”. The authors further remark that some of the tapetal cells are “scarcely distinguishable from pollen mother cells except by their position. The presence of such transitional forms between pollen mother cells and tapetal cells probably accounts for the occasional occurrence of synizesis in such cells”. They, however, do not state whether an actual conversion of spore mother cells to a tapetal function takes place. On the other hand Maheshwari (1934) finds in *Ophiopogon* that “all the sporogenous cells do not reach the mother cell stage. Some are abortive and may serve to nourish the remaining cells”. This indicates clearly that in addition to a normal tapetum, a tapetal function may also be taken over by some of the sporogenous cells. It is of interest to note here that a tapetal function may sometimes be ascribed even to the cells of the middle layers of the anther (Coulter and Chamberlain; 1903, Singh, 1936).

The presence of more than a single nucleus in the tapetal cells has been shown in a number of angiosperms. Cooper (1933), after a careful analysis, classifies the tapetal cells into three broad groups: (1) those in which the nucleus rarely, if ever, divides and the cells remain uninucleate; (2) those in which the nucleus divides mitotically once at about the time when the neighbouring pollen mother cells are in synizesis and the tapetal cells remain binucleate thereafter; and lastly (3) those in which the nucleus first divides as in the second group, after which further mitoses may occur resulting in plurinucleate cells. The tapetal cells in *Scaevola Lobelia* become quadri-nucleate usually, but sometimes are trinucleate on account of an irregular disposition of the spindles. The nuclei subsequently fuse again to form uninucleate cells just before the tapetal activity begins to decline. Raghavan (1938) has shown that a common condition of the tapetal cells in *Gynandropsis pentaphylla* is to form four nuclei which fuse subsequently.

Several authors have described the leptotene threads as double and presenting a beaded appearance on account of the intertwining of the two chromonemata. Koshy (1937) regards that diamond-shaped areas seen in favourable portions of the leptotene threads are suggestive of this feature. Naithani (1937), however, dismisses this for what appears to be an artefact caused by the fixing fluid; he states that in iron aceto-carmine preparations the leptotene threads are single and finely coiled in *Hyacinthus*. Similarly,
Huskins and Smith (1936) do not find double leptotene threads in Trillium; a linear arrangement of chromosomes is, on the other hand, evident here.

It is not possible to say definitely whether the leptotene threads in Scævola Lobelia, which present a beaded appearance and in which the clear diamond-shaped areas are seen, are really double with the two chromonemata coiled together, or, whether the picture obtained is merely an optical illusion induced by the fixing fluid. Unfortunately, other fixing fluids than Bouin's could not be employed at the time when the material was secured for study.

The structure of the chromosomes and the existence of spirally coiled strands composing them have been topics of much discussion recently. In specially favourable materials the precise nature of the major and the minor coils have been convincingly shown. It is not proposed to enter here into a discussion of this interesting aspect of study, because, the chromosomes are small and hence unsuitable for a detailed examination. It must suffice for the present to state that indications of a spiral nature are seen in some late diplotene pairs, which have lost the extra stain, either by continued destaining or on account of time. Further, the chromosome outline itself is very wavy suggesting the possibility thereby of the existence of coiled strands.

Darlington (1937) points out that two major types in the process of terminalization of chiasmata may be recognized. These are: (1) the Fritillaria-type with slight change of position and little or no reduction in the number of chiasmata through fusion; and (2) the Campanula-type, in which the movement is complete and results in terminalization of all the chiasmata. The plant under study here thus belongs to the second category, the chiasmata being regularly and completely terminalized towards late diakinesis. No irregularities are, therefore, seen in any of the pairs during the first anaphase separation.

Summary

1. The paper is a detailed account of the prophase changes in the nuclei of the microspore mother cells in Scævola Lobelia Linn.
2. The leptotene threads are beaded with clear diamond-shaped areas. It is therefore probable that the threads are double.
3. There are eight diplotene pairs and these are of different lengths. The nature and number of the chiasmata in these pairs are followed.
4. The late diplotene chromosomes show the presence of two coiled strands in specially favourable preparations.
5. The haploid number of chromosomes is determined from several countings to be eight; this number also corresponds to the number of diplotene pairs.
6. After the second division in the mother cells, the microspores are formed which show the typical tetrahedral arrangement.

7. The pollen grains are binucleate at the time of liberation from the anther locules.

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LITERATURE CITED


