

A CONTRIBUTION TO THE LIFE-HISTORY OF *VAHLIA VISCOSA*, ROXB., AND *VAHLIA* *OLDENLANDIOIDES*, ROXB.

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I. Introduction

THE family Saxifragaceæ is a fairly well worked family. It must be described as a heterogeneous family for the various genera of this natural order exhibit a variety of characters, and the systematic position of some of the genera has been questioned; for example, Pace (1912) having studied the life-history of a few species of *Saxifraga*, *Parnassia palustris* and *Drosera rotundifolia*, is of the opinion that *Parnassia* has greater affinities to *Drosera* than to *Saxifraga* and concludes that it should be included in the Droseraceæ. Dahlgren (1930) gives an account of the development of the endosperm in a number of

genera of the Saxifragaceæ and draws a scheme of endosperm development in five genera, where he distinguishes three important types of endosperm development, namely, nuclear endosperm, cellular endosperm and an intermediate type in which both kinds of endosperm development are combined. Chapman (1933) gives a summary of the more important of the work already done in the family. In 1933, Mauritzon made one of the best contributions to our knowledge of the development of the embryo-sac, endosperm and embryo in a number of genera of the Saxifragaceæ. In a critical study of the closely allied families of Crassulaceæ and Saxifragaceæ, he classifies the various genera of the Saxifragaceæ into two broad groups, the "Krassinucellate" and the "tenuinucellate", on the basis of the nature of the nucellus. Among the tenuinucellate, there are two groups, those in which there is a single layer of nucellar cells and those in which the nucellus is two cells thick just above the megaspore mother cell. He found that some genera were characterised by the possession of a single integument, while the others had two integuments. He also describes the nature of the ovary and ovule, and gives the developmental stages of the embryo-sac, the endosperm as also the embryo in a number of genera. Like Dahlgren (1930), Mauritzon (1933) also groups the various genera investigated till then under three heads, mentioned by Dahlgren (1930), the basis of grouping being the nature of endosperm development.

So far as we are aware, the work on the genus *Vahlia* is very meagre. Skovsted (1934) gives the haploid chromosome number of *Vahlia oldenlandioides* as six, which number is confirmed in the present investigation. Mauritzon (1933) has given a few stages in the development of the embryo-sac, the endosperm and the embryo in *Vahlia oldenlandioides*. No reference could be found in the available literature to any study of the species *Vahlia viscosa*, Roxb. The present paper describes the entire life-history of *Vahlia viscosa*, and also that of *V. oldenlandioides*, for purposes of comparison.

II. Material and Method

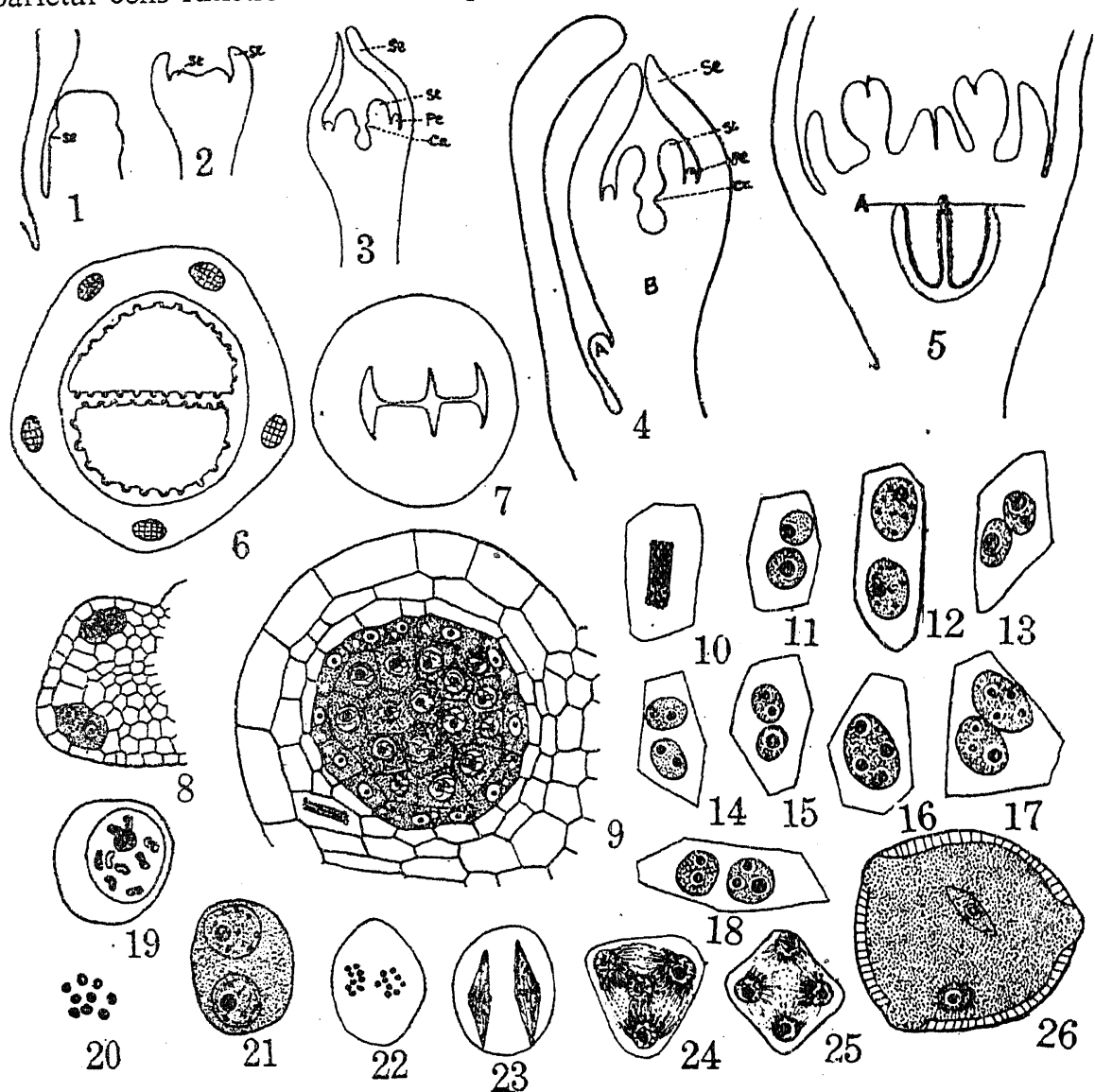
Plants of *Vahlia viscosa* and *V. oldenlandioides* were grown in the University Botanical Gardens, Annamalainagar. Different stages of ovary were fixed in corrosive sublimate formalin-Acetic-Alcohol fixative. For determining the chromosome number, flower buds after acetocarmine examination (to see whether they showed pollen mother cell division stages) were fixed in hot corrosive sublimate-formalin-Acetic-Alcohol fixative. Sections were cut at thicknesses varying between 6 and 14 microns. All the materials were stained in Haidenhain's Iron-alum Hæmatoxylin.

III. *Vahlia viscosa* Roxb.

(a) *Floral Organogeny*.—Flowers occur usually in pairs (Fig. 4, A and B) in the axils of leaves. Each flower is initiated as a small conical protuberance (Fig. 4, A). The sepals are the first to be initiated. Each sepal arises as a fold from the sides of the conical body (Fig. 1, *se*). The flower primordium, which to begin with, has a convex free end, at the time of the differentiation of the sepals becomes flattened and in later stages becomes progressively depressed, in the centre. The next floral organ to be initiated is the stamen and not the petal. The stamens arise as protuberances from near the rim of the depression or cavity which is being formed in the centre of the free end of the thalamus (Fig. 2, *st.*). The primordia of the petals appear between the stamens and the sepals (Fig. 3, *pe*). By this time, the depression in the free end of the thalamus is very deep. This is the ovarian cavity and from its sides near the top, two protuberances arise (Fig. 3, *ca*). These are the placenta. They grow downwards into the ovarian cavity into which they hang. When they are fully formed, the ovules appear as papillate protuberances from all round the two placentas. There can be no doubt that the gynæcium is made up of the fusion of two adjacent carpellary margins. This is quite clear when transverse sections are taken of very young gynæcia (Fig. 7) where the bi-carpellary nature of the ovary is quite evident especially at the top. The two carpel ends have not fused completely so that their individuality is unmistakably clear. At a later stage, they fuse together to form a single-celled ovary with two parietal placentæ (Fig. 6). On account of the peculiar disposition of the placentas, the latter appear as two oval bodies unconnected with either one another, or with the wall of the ovary, if a transverse section of the ovary is taken in the middle region. That the gynæcium is bi-carpellary and the placentation parietal, there can be no doubt about, as sections near the top of the ovary reveal the clear fusion of the adjacent carpellary margins. It is likely that the two carpellary margins by whose fusion the parietal placentas are primarily formed, have failed to form any such normal placentas except at the top of the ovary where on account of the massiveness of the placentas so formed and the concentration in one region, they hang as it were into the ovary cavity. In Fig. 5, a transverse section taken at level A, may reveal a position as depicted in Fig. 7, but a section taken lower down will present Fig. 6.

(b) *Microsporogenesis*.—Transverse sections of very young anthers reveal the primary archesporium. The primary archesporium is hypodermal and usually consists of a plate of two cells (Fig. 8). The primary wall cell cuts off towards the periphery a layer of primary parietal cells and a layer of primary sporogenous cells towards the interior. The primary parietal

cell again divides twice periclinally. As a result, the anther wall including the epidermis becomes four cells thick (Fig. 9). The innermost layer of the parietal cells functions as the tapetum. The tapetal cells are of differing

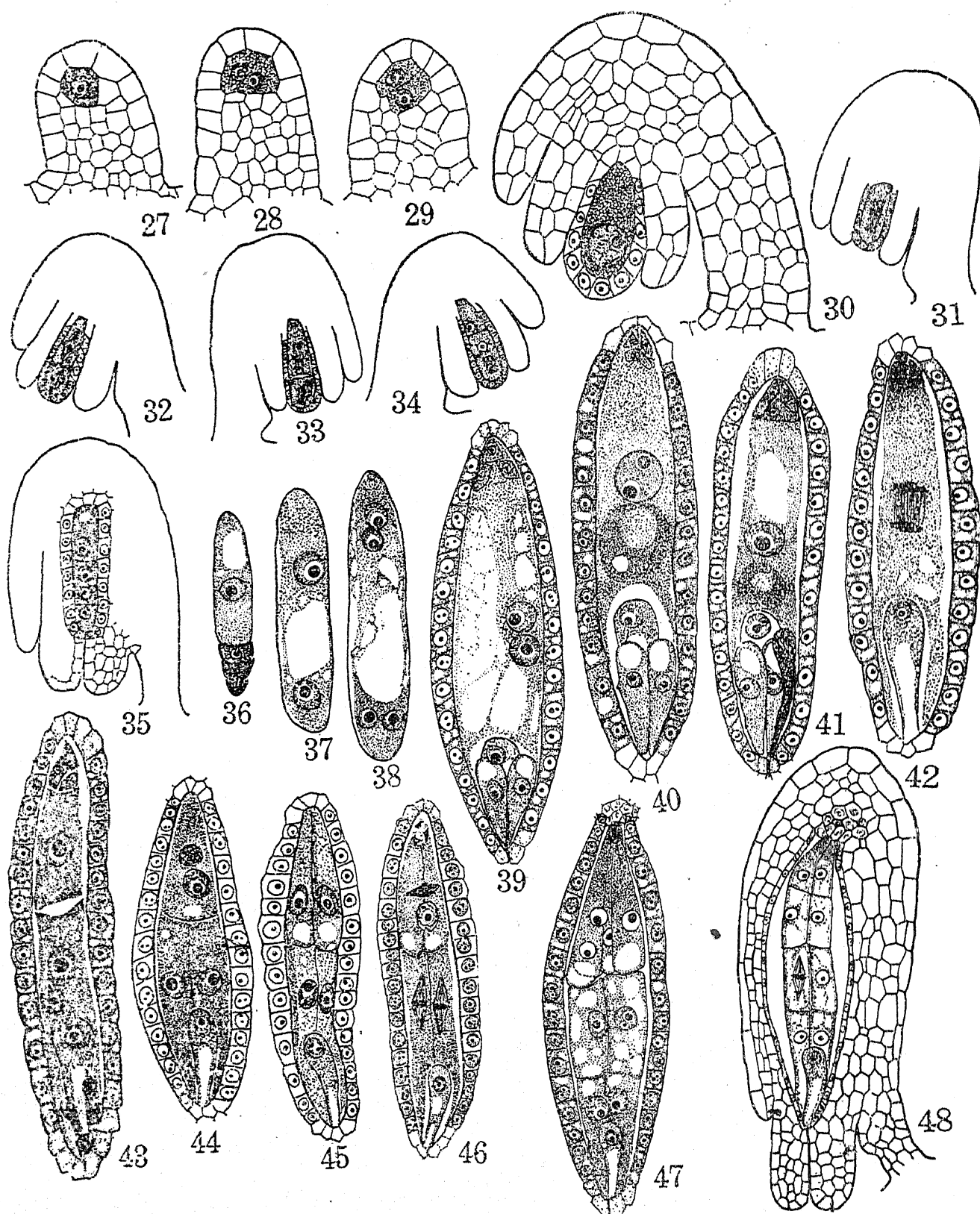


FIGS. 1-26. *Vahlia viscosa* Roxb.

Fig. 1. Origin of sepal at *se*. $\times 150$. Fig. 2. Origin of stamens at *st*. $\times 150$. Fig. 3. Origin of petal at *pe*. $\times 150$. Fig. 4. Shows the origin of two flowers A. and B in the axil of a leaf, and also that of the carpels at *ca*. $\times 150$. Fig. 5. L. S. of ovary showing the pendulous parietal placentas. $\times 150$. Fig. 6. T. S. of ovary showing uni-locular and bi-carpellary ovary. Five vascular strands are seen along the fruit wall. $\times 150$. Fig. 7. T. S. of very young gynæcium showing the bi-carpellary nature of the ovary. $\times 150$. Fig. 8. Hypodermal archesporium of two cells in the anther. $\times 800$. Fig. 9. Shows the anther-wall which is four cells thick, the innermost of which is the tapetum. $\times 1500$. Fig. 10. Mitotic division of the tapetal nucleus. $\times 2200$. Fig. 11. Bi-nucleate tapetal cell. $\times 2200$. Figs. 12-18. Various stages in the formation of the pluri-nucleate tapetal cells. $\times 2200$. Fig. 19. Pollen mother-cell nucleus in diakinesis; nine rod bivalents are present. $\times 2200$. Fig. 20. Metaphase I, $n = 9$. $\times 2200$. Figs. 22 and 23. Polar and side view respectively of Metaphase II. $\times 2200$. Figs. 24 and 25. Tetrahedral and isobilateral arrangement of the pollen tetrads. $\times 2200$. Fig. 26. Two-celled mature pollen grain. Note the three germ pores. $\times 2200$.

sizes and stain brightly, and to begin with are uni-nucleate (Fig. 9). Often small vacuoles appear in the tapetal cells. The pollen mother cells which are closely packed together are surrounded by the tapetal layer. When the pollen mother cells are in the early prophase, the nuclei of the tapetal cells divide and become bi-nucleate (Fig. 11). This division is distinctly mitotic (Fig. 10); the mitotic nature of the division of the tapetal nucleus has been observed in a number of families like Capparidaceæ (Raghavan, 1938), Scrophulariaceæ (Srinivasan, 1940), Acanthaceæ (Rangaswamy, 1941), etc. This is followed by a number of nuclear divisions and their immediate fusion, as a result of which the tapetal cells become pluri-nucleate (Fig. 15). Various stages in the formation of the pluri-nucleate condition have been noticed (Figs. 12–18). The possible significance of this has been discussed recently by Raghavan and Srinivasan, A. R. (1941). Fig. 19 represents the pollen mother cell nucleus in the diakinesis stage. Nine bivalents, all of which are of the rod kind can be clearly seen; two of them are attached to the nucleolus (Fig. 19). In Fig. 20 is represented the polar view of metaphase I, and nine bivalents are seen. Anaphasic separation is normal and the two groups of chromosomes as soon as they reach the poles, organise themselves into interkinesis nuclei. The nucleolus appears and the chromosomes which are more or less uniformly spaced are connected by thin strands (Fig. 21). In Fig. 22 is shown the second metaphase polar view. There are two groups of nine univalents each. Fig. 23 shows the side view of the second metaphase. The tetrads are formed by cell plate formation. They may be either iso-bilateral (Fig. 25) or tetrahedral (Fig. 24). At the time of shedding, the pollen grains are two-celled (Fig. 26). There are three germ pores.

(c) *Megasporogenesis*.—The ovary of *Vahlia viscosa* is bi-carpellary and uni-locular and the two parietal placentæ are pendulous and hang into the ovarian cavity (Fig. 5). The numerous anatropous ovules are arranged all around the placentas. At a very early stage, a hypodermal archesporial cell is differentiated (Fig. 27). Sometimes a plate of two archesporial cells is also found (Figs. 28 & 29). They may be either one below the other (Fig. 29) or side by side (Fig. 28). Multicellular archesporia are not uncommon in the Saxifragaceæ. Pace (1912) in *Parnassia palustris* reports the presence of archesporial plates composed of two, three or four hypodermal cells. The four cells were arranged in a linear row. Multicellular hypodermal archesporia have also been reported in *Jamesia americana* (Mauritzon, 1933). No wall cell is cut off by the archesporial cell. It begins to increase in size and functions directly as the megaspore mother cell (Fig. 30). Thus the genus *Vahlia* belongs to the “tenuinucellate”



FIGS. 27-48. *Vahlia viscosa*, Roxb.

Fig. 27. Hypodermal archesporium. $\times 1500$. Figs. 28 and 29. Two kinds of two-celled archesporial plate. $\times 1500$. Fig. 30. Megaspore mother cell invested by the nucellus. $\times 1500$. Fig. 31. Heterotypic division of the M.M.C. $\times 350$. Figs. 32-35. Various stages in the formation of the linear tetrad. $\times 350$. Figs. 36-39. One, two, four, and eight-celled embryo-sac.

In the 8-nucleate embryo-sac, the tapetal tissue does not surround the embryo-sac completely. $\times 1500$. Fig. 40. The polar nuclei are fusing; the synergids with the "synergidenhaken". Note the darkly-staining spherical body between the egg-cell and the polar nuclei. $\times 1500$. Fig. 41. Fertilization. The spherical body is present here also. $\times 1500$. Fig. 42. Division of the endosperm nucleus. $\times 1500$. Fig. 43. Two-celled endosperm. Note the dark body in the micropylar endosperm cell and the persisting antipodals. $\times 1500$. Fig. 44. Longitudinal division of the micropylar endosperm nucleus. In this case, the dark body is present in the chalazal chamber. $\times 1200$. Fig. 45. Four-celled endosperm. $\times 1200$. Fig. 46. Transverse division of the two micropylar endosperm nuclei and the longitudinal division of one of the two chalazal endosperm nuclei. $\times 1200$. Fig. 47. Shows four chalazal, two middle and two micropylar endosperm cells. $\times 1500$. Fig. 48. Ovule showing later stage in the development of the endosperm and degenerating tapetum. $\times 750$.

Saxifragaceæ. In the "Krassi-nucellate" genera of the Saxifragaceæ, the archesporial cell cuts off a parietal cell which builds up a massive nucellus. When there is a plate of two archesporial cells, one of them alone functions, for no case of two megaspore mother cells or two tetrads or two embryo-sacs was noticed in hundreds of sections examined. Chapman (1933), however, found that the occurrence of two embryo-sacs in the same ovule was not rare in *Saxifraga virginensis*. In all these cases, the extra embryo-sacs were developed from a megaspore resulting from the division of a second megaspore mother cell. In three out of five cases, each of the embryo-sacs was surrounded by its own nucellus, and in these, the embryo-sac was in the two or the four-nucleate condition. In the other two cases, he found two megaspore mother cells, one of which was in the prophase of the first division, while in the other, the nucleus was in the metaphase of the first division. In one case, the two megaspore mother cells lay side by side, while in the other, they were separated by two or three layers of nucellus. This kind of archesporium which functions directly as the megaspore mother cell has been reported in *Jamesia americana*, *Philadelphus coronarius* (Mauritzon, 1933) and in *Parnassia palustris* (Pace, 1912). Just at the time, when the primary archesporium increases in size, to assume the functions of the megaspore mother cell, the primordia of the integuments arise. The inner integument is the first to be initiated, while the outer soon follows the inner. The inner integument is longer than the outer and it alone takes part in the formation of the micropyle (Fig. 35). As the integuments grow, the ovule curves to assume its anatropous nature (Fig. 30). At this stage, the megaspore mother cell could be seen to be invested almost to its base by a layer of cells, the nucellus (Fig. 30). This nucellus is derived by repeated anticlinal divisions of the epidermal cells just above the primary archesporium. In the Saxifragaceæ both single and two integumented ovules occur. The genera *Ribes*, *Vahlia*, *Brexia*, *Parnassia*, etc., have two integuments, while the genera *Saxifraga*, *Kirengeshoma*, *Hydrangea*, etc., possess a single integument. With the development of the megaspore mother cell, the cells of the

nucellus get flattened and finally disorganise and disappear completely, in the early stages of the development of the embryo-sac. When the megaspore mother cell has increased in size considerably, the heterotypic division sets in, as a result of which a dyad is formed (Fig. 32). Fig. 31 represents the telophase of the heterotypic division. The cells of the dyad undergo the homotypic division to give rise to a linear tetrad (Fig. 35). The dyads do not divide simultaneously. Figs. 32 to 34 show the various stages in the formation of the tetrad. In Figs. 32 and 33, the dyad cells are dividing simultaneously. In Fig. 32, the two nuclei are at metaphase. In Fig. 33, while the chalazal dyad is in the metaphase of the division, the micropylar dyad cell is in the telophase of the same division. In Fig. 34, the chalazal dyad has divided to form two cells, while the micropylar cell is still in the anaphase of the first division. Such a non-simultaneous division of the dyads has been recorded in *Parnassia palustris* (Pace, 1912). Here, while the chalazal dyad cell has only formed the chromosomes, the micropylar cell has already formed the spindle for the second division. Though the linear arrangement of the tetrads is more common in the Saxifragaceæ, T-shaped tetrads have also been reported in some cases. Mauritzon (1933) reports the occurrence of T-shaped tetrads in *Bergenia crassifolia*, *Ribes aureum*, *Tiarella cordifolia*, *Heuchera sanguinea* and Chapman (1933) in *Saxifraga virginensis*. The chalazal megaspore is the functional one and the rest degenerate (Fig. 36). The functional megaspore divides to give rise to the 2-nucleate embryo-sac (Fig. 37). The four and the eight-nucleate embryo-sacs (Figs. 38 & 39) are formed in the usual manner. Fig. 39 shows the mature embryo-sac, where the egg apparatus has been organised. There are two prominent synergids, an egg cell, two polar nuclei situated usually in the middle of the embryo-sac and three antipodals towards the chalazal end. The filiform apparatus described by Pace (1912) as being usual in *Saxifraga virginensis* was not evident in the present case. The "Synergidenhaken" however are seen, though not very markedly, as broadened bracket-like bases of the synergids (Fig. 40). In the Saxifragaceæ, these structures have been reported in *Hydrangea petiolaris*, *Francoa appendiculata*, *Ribes stenocarpum*, *R. nigrum*, *R. grossularia*, *Mitella nuda* (Maurtizon, 1933), etc. Fig. 40 shows the two polar nuclei fusing in the centre. The two nucleoli can still be seen. The secondary nucleus is the largest and most prominent nucleus in the embryo-sac.

The mature embryo-sac is roughly elliptical in shape. The other genera of the Saxifragaceæ have embryo-sacs of differing shapes (Maurtizon, 1933). The embryo-sac is long, narrow and cylindrical in *Francoa appendiculata*; highly enlarged in the micropylar region, while the chalazal portion is

narrow, wherein the antipodals are situated as in *Heuchera pubescens* and *Mitella nuda*; more or less elliptic as in *Itea virginica*, *Escallonia macrantha*, *Parnassia ovata*; the chalazal portion of the embryo-sac may be bent at an angle to the micropylar portion as in *Hydrangea petiolaris*. In *Polysoma ilicifolium*, the embryo-sac is obovate in shape, the chalazal end being broad and blunt, while in *Ribes missouriense*, the sides of the embryo-sac dilate and grow towards the chalazal end of the ovule like a haustorium.

At about this stage, when the embryo-sac is fully organised and the polar nuclei have fused, a spherical, darkly-staining nucleus-like body makes its appearance just above the fusion nucleus. This appears to be a feature of constant occurrence, for in about sixty or seventy embryo-sacs examined, we found this body always present. It cannot, however, be a nucleus since there appears to be no definite nuclear membrane and also there is no nucleolus. This body persists till after fertilization. In Fig. 41, where the remains of the pollen tube are seen and the egg cell is undergoing fertilization, we find this spherical body in a line with the fusion nucleus and the fertilized egg. In Fig. 43, where fertilization of the egg cell has been completed as could be seen from the degenerated synergids and where the endosperm has formed a 2-celled structure, we find this body again. In Fig. 44, which is a later stage showing 3-celled endosperm, we find this body below the chalazal endosperm nucleus. Later than this stage we have not met with this body. We are not at present able to offer any interpretation as to the exact nature of this spherical body.

(d) *Tapetum*.—As a result of the breakdown of the single layer of nucellus investing the megaspore mother cell, the innermost layer of the inner integument comes into direct contact with the sides of the embryo-sac. This layer of cells, the tapetum, becomes conspicuous on account of the regularly arranged rectangular cells and they soon come to possess rich cell contents and hence stain rather darker than the other cells of the integument. Often, however, small vacuoles could be noticed in the tapetal cells. The cross walls are oblique. The tapetum is thus of integumentary origin and its differentiation from the inner integument commences simultaneously from either end of the embryo-sac. The tapetal cells are uni-nucleate though often bi-nucleolated (Fig. 39). Bi-nucleate tapetal cells have been occasionally recorded in the Solanaceæ (Bhaduri, 1932) and in the Orobanchaceæ (Srivastava, 1939). The tapetum does not surround the entire embryo-sac. At the chalazal and micropylar ends, only ordinary cells are found. A distinct tapetal tissue surrounding the embryo-sac has been reported in various genera of the Scrophulariaceæ (Srinivasan, 1940), Solanaceæ (Bhaduri,

1935), Labiateæ (Billings, 1909), Lobeliaceæ (Kausik, 1938), Orobanchaceæ (Srivastava, 1939), Verbenaceæ (Tatachar, 1940), and in many other sympetalous families, as also in Crassulaceæ (Mauritzon, 1933) of the polypetalæ and in *Parnassia palustris* (Pace, 1912), *P. ovata* and *Brexia madagascariensis* of the Saxifragaceæ (Mauritzon, 1933). In *Escallonia rubra*, and *Hydrangea petiolaris* also belonging to the Saxifragaceæ, the tapetal tissue is confined to the chalazal half, which is often bent at an angle to the micropylar half (Mauritzon, 1933). The function of the tapetum is essentially nutritive. For, as the endosperm in the embryo-sac increases in size, the tapetal tissue gradually becomes thinner and thinner (Fig. 48) and finally disappears.

(e) *Fertilization*.—Fig. 41 shows the male nucleus about to fertilize the egg. The darkly staining pollen tube enters the embryo-sac through the micropyle. The male cell would appear to be spherical. Though vermiform and spiral-shaped male cells are by far the commonest in Angiosperms, spherical cells have however been occasionally reported, e.g., Weinstein (1926) in *Phaseolus vulgaris*, Madge (1929) in *Viola odorata*, Newman (1934) in *Acacia Baileyana* Raghavan (1937) in *Cleome chelidonii*, and Raghavan and Srinivasan (1941) in *Ilysanthes parviflora*. The male nucleus and the egg nucleus seem to be in a resting condition at the time of contact. Such a condition is not only common in Angiosperms but also in the Coniferales (Guilliermond, 1933) and some Cycadales (Lawson, 1926). No phylogenetic significance can be attributed to this, as this phenomenon is found in such widely separated families as Oenotheraceæ (Ishikawa, 1918), Hydrocharitaceæ (Wylie, 1923), Orchidaceæ (Pace, 1907), Capparidaceæ (Raghavan, 1937), and Scrophulariaceæ (Raghavan and Srinivasan, V.K., 1941). The synergids are ephemeral and degenerate soon after fertilization. The antipodals often show a tendency to persist, though in a rather degenerated form, and are to be seen in embryo-sacs, in which the endosperm has become two to four-celled (Figs. 43 and 46). In the Saxifragaceæ, antipodal haustorium is known to occur in *Kirengeshoma palmata* (Mauritzon, 1933); though all the three antipodals which are arranged one above the other in a linear fashion persist, the one towards the extreme chalazal end is elongated and is haustorial in its function.

(f) *Endosperm*.—The fusion endosperm nucleus is the largest nucleus in the post-fertilization embryo-sac. The position of the endosperm nucleus is always in the middle of the embryo-sac. In the other genera of the Saxifragaceæ, the position of the endosperm nucleus varies considerably. In *Mitella pentandra* (Dahlgren, 1930), the endosperm fusion nucleus is more towards the antipodal end. The fusion nucleus undergoes a period of rest before it divides. The first division is transverse and is accompanied by

wall formation. Fig. 42 shows the fusion nucleus in the anaphase of the transverse division. The transverse wall divides the embryo-sac into two more or less equal halves (Fig. 43). In the other genera of the Saxifragaceæ that exhibit cellular endosperm, the first wall is also transverse, though the two endosperm chambers that result in the embryo-sac are not equal as in the present case. Usually, in these, the chalazal endosperm chamber is considerably smaller than the micropylar one. Such a difference in size of the two cells of the two-celled endosperm stage has been figured for *Mitella pentandra*, *Boykinia occidentalis* (Dahlgren, 1930), *Boykinia Jamesii*, *Bergenia ligulata*, *Saxifraga micranthidifolia*, *Tiarella polyphylla* and *Ribes bureiense* (Mauritzon, 1933). Of the two endosperm cells thus formed, the micropylar one is the next to divide. This division is longitudinal followed by wall formation (Fig. 44). Following closely on this, the chalazal cell also divides longitudinally (Fig. 45). As a result of these two divisions, four endosperm cells are formed in the embryo-sac. This bears a close resemblance to the sequence of division of the endosperm nucleus in a few genera of the Scrophulariaceæ (Srinivasan, 1940). In the other genera of the Saxifragaceæ, however, the second and the third divisions are not longitudinal as in *Vahlia viscosa*. The second division is frequently transverse resulting in a row of three cells as in *Mitella pentandra* (Dahlgren, 1930). The two micropylar endosperm cells (Fig. 46) then divide transversely followed by wall formation. As a result, six endosperm cells are formed in the embryo-sac. The two cells in the middle of the embryo-sac by repeated divisions form the cellular endosperm tissue. The two chalazal and micropylar cells also cut off cells towards the centre of the embryo-sac and contribute to the endosperm. The two chalazal cells often undergo a longitudinal division resulting in four cells arranged in two juxtaposed tiers one tier below the other (Fig. 47). These cells become very rich in cytoplasm and hence stain brightly and the cells abutting on the chalazal end of the embryo-sac begin to exhibit signs of degeneration. These four chalazal endosperm cells thus appear to be haustorial in function, though they do not show any well-marked growth or haustorial protuberance penetrating the tissue around it. Though the endosperm cells towards the micropylar end also take a deep stain, they do not seem to be haustorial as the adjoining tissue does not show any signs of disintegration. As the endosperm tissue increases in size in the embryo-sac, the tapetal layer surrounding the embryo-sac becomes thinner and thinner and in very late stages, it completely disappears. This is as it should be because the tapetum is essentially nutritive. As the endosperm develops rapidly, it absorbs nutrition required for its growth from the tapetum, which consequently becomes shrivelled up gradually.

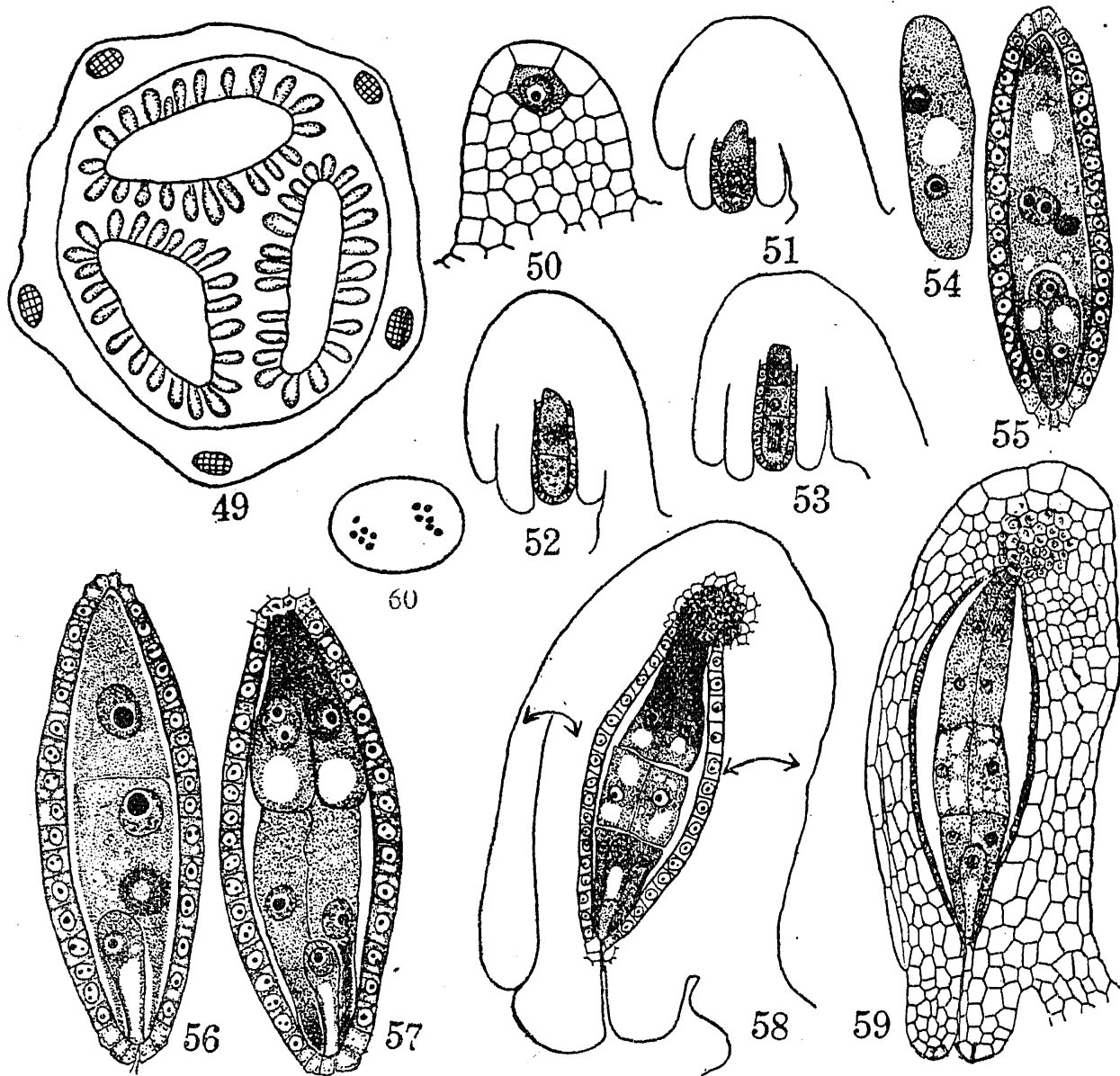
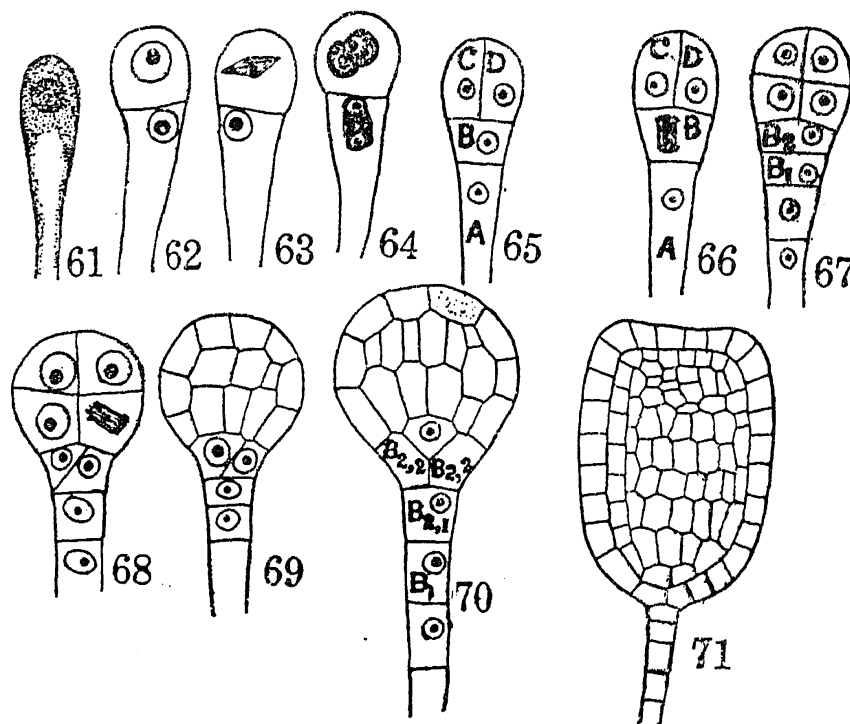
FIGS. 49-60. *Vahlia oldenlandioides*, Roxb.

Fig. 49. T. S. of ovary showing three placentas. $\times 75$. Fig. 50. Hypodermal archesporium. $\times 1500$. Fig. 51. M.M. cell with nucellus. $\times 350$. Fig. 52. Dyad in division showing six chromosomes. $\times 350$. Fig. 53. Formation of the linear tetrad. $\times 350$. Fig. 54. Two-nucleate embryo-sac. $\times 1500$. Fig. 55. Eight-nucleate embryo-sac, the polar nuclei fusing with the darkly staining body situated near it. $\times 1200$. Figs. 56 and 57. Two- and four-celled endosperm. $\times 1200$. Figs. 58 and 59. Ovules showing later stages of endosperm development. $\times 750$. Fig. 60. Metaphase II, $n = 6$. $\times 2200$.

(g) *Embryo*.—The oospore (Fig. 61) undergoes a long period of rest before it begins to develop into the embryo. The oospore divides long after the endosperm nucleus has divided. A stray case where in one embryo-sac, the oospore divides before the endosperm is reported by Pace in *Parnassia palustris* (1912). Here, he observed a 5-celled embryo with the endosperm nucleus still undivided and somewhat amœboid in shape. After a considerable amount of endosperm tissue has been formed, during which

time, the oospore has elongated to some extent, it divides. Consequently the oospore is surrounded by the endosperm tissue. The first division of the oospore is transverse and is immediately followed by a cross-wall (Fig. 62). Of the two resulting cells, the lower one, *i.e.*, the one farther from the micropyle, gives rise to the embryo proper, and the upper, to the suspensor. The lower or apical cell is the next to divide. This division is anticlinal (Fig. 63). Following closely upon this division, the upper one also divides, but transversely, both the divisions being followed by the formation of cross-walls (Fig. 64). Within the Saxifragaceæ, a similar behaviour of the 2-celled embryo has been figured in *Parnassia palustris* (Pace, 1912). In this also, while the apical or lower cell divides longitudinally, the basal or upper cell divides transversely. There is however some difference in the timing of the two divisions between the two-celled embryo in *Parnassia* and in the present case. For, while in *Vahlia viscosa*, the lower (embryonal) cell divides before the basal cell, in *Parnassia palustris*, from the figure given by Pace (1912), it would appear that the upper cell begins to divide a little earlier than the apical cell. In Fig. 64, the lower cell has divided while the upper is in the late telophase of the transverse division. As a result of these two divisions, a four-celled pro-embryo is formed, in which the two lower cells are placed side by side, while the upper two are placed one above the other (Fig. 65) and as such the 4-celled pro-embryo is only three cells long. This type of arrangement of the four cells of the pro-embryo is common in families like Cruciferae, Ranunculaceæ (Souges, 1913, 1919), Capparidaceæ (Raghavan, 1937), etc. In the other type, the four cells of the pro-embryo are arranged in a linear fashion. Such an arrangement of the four cells of the pro-embryo is characteristic of the Rubiaceæ (Raghavan and Rangaswamy, 1941), Solanaceæ (Souges, 1922), Leguminosæ (Cooper, 1933), Orobanchaceæ (Srivastava, 1939), Scrophulariaceæ (Srinivasan, 1940), and in the Acanthaceæ (Rangaswamy, 1941). In the Saxifragaceæ, both types of arrangement of the four cells in the pro-embryo are met with. For instance, in *Ribes divaricatum*, in *Astilboides tabularis* and in *Heuchera sanguinea* (Mauritzon, 1933), the four-celled pro-embryo is linear, while in *Parnassia palustris* (Pace, 1912), the arrangement of the four-celled embryo is similar to that found in the present investigation. Besides these two types of arrangement, often a linear pro-embryo of five or more cells in length is also to be found as in *Boykinia tellimoides* (Dahlgren, 1930), and *Tiarella polyphylla* (Mauritzon, 1933). The four-celled embryo stage is an important one, as each one of these four cells gives rise to a definite region in the mature embryo. For purposes of description, these four cells beginning from the uppermost or basal cell will be designated A, B, C and D. The cell B is the

first to divide among the four cells of the pro-embryo. It divides by a transverse wall (Fig. 66) into B_1 and B_2 , as a result of which the suspensor becomes three cells long (Fig. 67). Often this division is followed by an oblique cross-wall as shown in Fig. 68. The suspensor becomes four cells long



FIGS. 61-71. *Vahlia viscosa*, Roxb.

Various stages in the development of embryo. Figs. 61-70. $\times 1500$. Fig. 71. $\times 1100$.

by another transverse division of the cell A. The cell B_2 divides transversely into two cells $B_{2,1}$ and $B_{2,2}$ of which the latter is the hypophysis. The cell $B_{2,2}$ cuts off a cell which abutts into the embryonal sphere and is itself divided into two cells by a vertical wall (Fig. 70). Figs. 69 and 70 are those of later stages in the embryo development and show the differentiation of the primary tissues, dermatogen, periblem and plerome. In the mature embryo, the suspensor is about 5 to 6 cells long and is uni-seriate (Fig. 71). Multi-seriate and massive suspensors especially at the base, are a common feature of most members of the Saxifragaceæ. Multi-seriate suspensors have been recorded in *Mitella dyphylla*, *Sullivantia sullivantii*, etc., and in *Ribes aureum*, the suspensor is very massive and irregular in shape.

IV. *Vahlia oldenlandioides*, Roxb.

The two species of *Vahlia* investigated here, are characterised by parietal placentation. The placentas are pendulous and hang from the roof of the gynæceum into the ovarian cavity as has already been described. In *V. oldenlandioides*, however, often three parietal placentas and three styles

are to be found, and Fig. 49 shows the transverse section of such an ovary. The development of the ovule, embryo-sac, the endosperm and of the embryo bears a striking resemblance to that already described for *V. viscosa*. The primary archesporium which is hypodermal may consist of a single cell (Fig. 50) or a plate of two cells. This species of *Vahlia* is also characterised by the possession of two integuments, the inner of which alone takes part in the organisation of the micropyle. The nucellus is also similar to that in *Vahlia viscosa*. Fig. 52 represents the dyad. The nuclei of the dyad are in the metaphase of the homotypic division and in the miropylar dyad, the haploid chromosome number of six is clearly seen. A linear tetrad is formed (Fig. 53), the chalazal one of which develops into the mature embryo-sac in the usual manner (Fig. 55). Fig. 54 shows the bi-nucleate embryo-sac. The mature embryo-sac bears a close resemblance to that of *V. viscosa* and is surrounded by a tapetum of integumentary origin. The tapetal cells are uni-nucleate. Even in this species the darkly staining spherical body described for the other species was found to be a feature of constant occurrence. The endosperm cells towards the chalazal end have a haustorial function as in the other species already described.

Thus we find that the life-histories of the two species of *Vahlia* are very similar and it would appear that the genus *Vahlia* is characterised by the possession of two or three parietal and pendulous placentas, ovules with two integuments and a single layer of nucellus which degenerates very soon giving place to a tapetal layer of integumentary origin, the tapetal cells being uni-nucleate. The chalazal megaspore of a linear tetrad always forms the embryo-sac, which is normal, the degenerated antipodals often persisting till the two-celled endosperm stage. Endosperm is cellular, and in both the species, the plan of cellular endosperm development is different from the other genera of the Saxifragaceæ so far studied, but is similar among themselves. In the four-celled pro-embryo, the two apical cells are placed side by side while the two basal cells are one over the other and the suspensor is uni-seriate and about 5 cells long.

The haploid chromosome number has been determined to be 6 and this finding corroborates the number already recorded by Skovsted (1934). Fig. 60 is that of M II in polar view and shows two groups of six univalents each.

V. Discussion

(a) *The Endosperm*.—The family Saxifragaceæ is interesting especially from the point of view of endosperm development. Within the family, three distinct types of endosperm development are found. Some genera are characterised by the cellular type ; in some others the free

nuclear type of endosperm development is the rule. The third or the "Helobiale type" is a combination of the cellular and the free-nuclear type of endosperm development. In this last mentioned type the first one or two divisions are followed by the formation of cross-walls while successive divisions produce free nuclei, wall formation commencing later. Of the two cells resulting from the first division, in one the development of the endosperm will be cellular, while in the other cell it is exclusively free-nuclear. Mauritzon (1933) has classified the plants investigated till his time into three groups on the basis of the nature of endosperm development. According to him, the genera *Brexia*, *Parnassia*, *Tetilla* and *Francoa* have the "nuclear type" of endosperm. The genera *Astilbe*, *Astilboides*, *Bergenia*, *Boykinia*, *Cardiandra*, *Decumaria*, *Deutzia*, *Heuchera*, *Hydrangea*, *Jamesia*, *Kirengeshoma*, *Lithophragma*, *Mitella*, *Peltiphyllum*, *Philadelphus*, *Ribes*, *Rodgersia*, *Tellima*, *Tiarella*, *Tolmiea* and *Vahlia* exhibit the "cellular type" of endosperm development, while the genera *Boykinia*, *Chrysosplenium*, *Mitella*, *Ribes*, *Saxifraga*, *Sullivantia* and *Tiarella* possess the "Helobiale type" of endosperm in which endosperm development is cellular to begin with but later becomes free-nuclear. There are, as will be seen from the examples cited, a number of cases, in which the two types of endosperm development are found in the same genus. For example, in the genus *Mitella*, the species *M. diphylla* exhibits the "Helobiale type" of endosperm development, while in *M. pentandra* its development is exclusively "cellular". Again in *Boykinia*, *B. aconitifolia*, *B. Jamesii* and *B. occidentalis*, show the cellular type of endosperm; *B. tellimoides*, however, is characterised by the "Helobiale type" of endosperm. Similarly in the genus *Tiarella* while some species show the "Helobiale type" of endosperm, a few other species exhibit "cellular" endosperm. Thus we find that in the Saxifragaceæ endosperm development varies considerably from genus to genus and even within the same genus, different species exhibit different type of endosperm development. In the two species of *Vahlia* investigated, cellular endosperm is the rule. Taking into consideration the cellular type of endosperm development in the Saxifragaceæ, we find that all the species showing cellular endosperm do not follow the same plan or sequence of divisions in the building up of the endosperm. Even here, we find that different genera show different plan or sequence of divisions in the formation of the endosperm tissue. In the Saxifragaceæ, among those exhibiting cellular endosperm the more usual method seems to be the formation of three endosperm cells by two transverse walls laid across the embryo-sac, and during the further development of the endosperm tissue a longitudinal wall is usually laid in the chalazal chamber to begin with,

and similar longitudinal walls are formed in the other two endosperm cells also. This type of cellular endosperm development has been noted by Mauritzon (1933) in *Astilboides tabularis*, *Mitella nuda*, *Bergenia ligulata*, etc., while in others like *Boykinia occidentalis* (Dahlgren, 1930), the second division takes place in the chalazal cell and is longitudinal. In the present investigation, the two species of *Vahlia* studied present an altogether different plan of endosperm development from the others. The first division is transverse, while the second division is longitudinal and takes place in the micropylar endosperm cell. The third division is also longitudinal and takes place in the chalazal cell. The two micropylar cells then divide transversely. Such a plan of division of the endosperm nucleus has not so far been reported in any other member of the Saxifragaceæ. A similar scheme of endosperm development has been found in *Dopatrium lobelioides*, *Stemodia viscosa* and *Vandellia crustacea* all belonging to the Scrophulariaceæ (Srinivasan, 1940).

While in the closely related family of Crassulaceæ (Mauritzon, 1933) haustoria of diverse origin, like megaspore haustorium, suspensor haustorium, chalazal and micropylar endosperm haustorium are common, in the Saxifragaceæ, however haustoria are of rare occurrence. In *Kirengeshoma palmata* (Mauritzon, 1933) antipodal haustoria are present. Micropylar haustorium of endospermal origin has been recorded by Mauritzon (1933) in *Corokia cotoneaster*, in which is figured a single uni-nucleate micropylar endosperm haustorium. Four uni-nucleate vermiform micropylar haustoria occur in *Philadelphus coronarius* (Mauritzon, 1933). The function of these haustoria is the usual one of supplying nutrition to the developing endosperm and embryo.

An effort was made to find out whether a correlation could be established between the division of the family on the basis of the type of endosperm development and the division of the family by taxonomists on morphological grounds. We also endeavoured to discover whether chromosome numbers that are known so far in this family could, in any way be employed for the elucidation of this. In this connection, we gathered most of the available data regarding this family, both morphological and cytological. Engler recognises seven sub-families and in trying to find out if a relationship could be established between the type of endosperm formation as available from previous work and this classification, we found that within each sub-family there occurred almost all the three types of endosperm development. In some cases, as in the genus *Ribes*, even within a genus, different types of endosperm development are reported. The statement given below will show this point clearly. For instance, in the sub-family Saxifragoidæ, *Vahlia* shows the "cellular type," so also *Astilbe*; *Parnassia*, shows "nuclear",

Chrysosplenium and *Saxifraga* show the "helobiale type" of endosperm development. The Hydrangeoideæ is a bit more uniform where the three genera about which information is available show the "cellular type" of endosperm but no generalisation is possible since we have no information about the other sixteen or seventeen genera comprised in this sub-family. In the tabular statement presented, only important genera included in the sub-families are shown; it will be seen that practically no information is available about the method of endosperm formation in the sub-families Petrostemoideæ, Escallonoideæ, and Baueroideæ. But the information available at hand shows clearly that there could be no question of the division of the family on the basis of the method of endosperm formation. The chromosome numbers of a comparatively large number of genera included in this family are known. But these are confined principally to the sub-families Saxifragoideæ, Hydrangeoideæ and Ribesioideæ. Some of the numbers known are noted against the respective genera. It could be seen that aneuploidy has played, presumably, a very important part in the evolution of the genera and that no support could be had from these numbers for tackling the problem cyto-taxonomically. We also endeavoured to see if the chromosome numbers and the type of endosperm development could in any way be related. For example, the species showing the "cellular type" of endosperm exhibit numbers: 6, 7, 8, 9, 13, 16, 18, 38, 39, 52, etc. Similarly, the "nuclear" forms exhibit 9, 10, 20 and those belonging to the "Helobiale type" show also a similar range of chromosome numbers: 8, 9, 11, 13, 16, 18, 20, 24, etc. It is obvious from these data that have been presented, that it may not be easy to classify the family easily, from these points of view. It has already been said that it is a rather heterogeneous family; the position of some members is even doubtful, as for example that of *Parnassia* already mentioned. If the problem is to be tackled cyto-taxonomically, it would be better to arrange the different genera and species according to the respective chromosomal series: those belonging to the 8 series, 9 series and so on. The genus *Deutsia* for instance would appear to fall in with the 13 series; so also *Philadelphus*. *Hydrangea* belongs to the 9 series. In *Saxifraga* we get an aneuploid series 8, 9, 11, 13, 18, and so on. Very likely, a more critical cytological examination may throw some light upon the basic number of the genus and also that of the sub-family. In this, phenomenon like secondary association coupled with some genetical data will undoubtedly play a very large part. It may be that there is a primary basic number from which there might have arisen other secondary basic numbers, each of which might have produced their own polyploid series. How these secondary polyploid series arose can again be inferred only by a cytological and genetical study. In this

way, if data are gathered, it may be possible to tackle the problem cytologically. Pending this, it can only be said that the family is indeed, in the words of C.B. Clarke (1879) "very difficult of definition".

I. *Saxifragoideæ*:

- H Saxifraga (6, 7, 8, 9, 13, 14, 15, 16, 18, 36, 39, 52, 65).
- Z Vahlia (6, 9).
- H Chrysosplenium (12, 24).
- Z Heuchera (7, 8).
- N Parnassia (9, 10).
- Z Astilbe (7).
- Z & H Tiarella (9).

II. *Francooideæ*:

- N Francoa (20).
- N Tetilla.

III. *Hydrangeoideæ*:

- Z Hydrangea (18, 36).
- Z Philadelphus (13).
- Z Deutzia (13, 39, 52, 65).

IV. *Pterostemonoideæ*:

- Pterostemon.

V. *Escallonoideæ*:

- Escallonia.
- Phylloma.
- Polysoma.
- Itea.

VI. *Ribesiodeæ*:

- Z & H Ribes (8, 16).

VII. *Baueroideæ*:

- Bauera.

N.B.—The numbers given in brackets on the right-hand side of the genera are the chromosome numbers prevalent in the respective genera, and the bold letters on the left-hand side denote the type of endosperm development found in the respective genera:

- Z = Cellular endosperm.
- N = Nuclear endosperm.
- H = Helobiale type of endosperm.

(b) *The Nucellus and the Tapetum.*—The family Saxifragaceæ is interesting not only from the point of view of endosperm formation but also from that of presence of the tapetum. In this connection, we have tried to gather available information regarding the tapetum, endosperm formation and the

nature of the nucellus and the possible correlation between these. Generally speaking, nuclear type of endosperm formation is associated with a massive nucellus. This is widely prevalent amongst the apetalæ and polypetalæ of Bentham and Hooker. In these, there is no tapetum formation. This statement does not mean, however, that this is a rigid rule. There are some families amongst this group of Angiosperms where we get reduced nucellus "Tenuinucellate" (single layer of nucellus) as for instance in Sarraceniacæ, Podostomacæ, Pittosporacæ, etc. There are also a few cases in this group, where the massive nucellate condition "Krassinucellate" is associated with cellular endosperm. All that the statement implies is that in the vast majority of the families comprised in this group, massive nucellus and nuclear endosperm coupled with the absence of any tapetum seems to be the rule. In the Sympetalæ, the reduced nucellus is the prevailing condition. As in the previous case, there are found a few families in which this reduced nucellate condition (tenuinucellate) is associated with nuclear endosperm for example, Gentianacæ, Apocyanacæ, Loganiacæ, Asclepiadacæ, Rubiacæ, Goodeniaceæ, etc. It is amongst this group, that we get the tapetum and it is in some of the families comprised in this group that the endosperm haustorium is prevalent. The pertinent question arises, whether there is any factor which governs the appearance of the tapetum. The tapetum such as occurs in the ovule, is almost always integumentary in origin, and must be regarded as nutritive in function even as the microsporangial tapetum. While there can be no doubt as to the nutritive character of the latter, because of its universal occurrence, the same cannot be said of the integumentary tapetum, because it is confined only to some families. In order, therefore, to find out its true role, and also if possible, the conditions under which it usually occurs, we tried to see if a correlation could be discovered between this and the other associated tissues like the nucellus and the endosperm. Such an investigation has revealed as has been indicated, the existence of some relationship from which could be drawn a few inferences which for the moment must obviously be regarded as tentative. The first observation of importance is that the tapetum almost always is integumentary in origin. That is, it occurs only where there is no parietal tissue. A possible inference from this is that where there is no massive (Krassinucellus) parietal tissue, the nutrition of the embryo-sac is presumably defective, however much its place may be taken up by the integument. Naturally, in order to strengthen the nutritive mechanism, the tapetal layer is present. Support to this can be gathered from the fact that the occurrence of the tapetum is mostly associated with cellular endosperm, many of which exhibit some type of haustorium or other. This means that because the

nutritive mechanism is not perfect, recourse has been taken to these supplementary devices, by which to make up for the deficiency. The next question is in what way does the tapetum discharge its nutritive role? If an analogy is to be established between this tapetum and the anther tapetum we must naturally look for the pluri-nucleate condition of the tapetal cells and the subsequent usage of this nuclear material for the nutrition of the embryo-sac. Though this multi-nucleate condition is widely prevalent so far as the anther-sac tapetum is concerned, this appears to be rather the exception than the rule so far as the integumentary tapetum is concerned. This leads to the question whether the tapetal cells directly contribute to the nutrition of the embryo sac or does the tapetal layer merely act as a sort of a liaison tissue, merely helping in the transference of nutritive material from the surrounding integumentary tissue. To our mind the latter alternative seems to be the more possible, for as has already been said, this tapetum occurs only where the massive (Krassi) nucellate condition does not exist. In this "Krassinucellate" condition, the embryo-sac is closely surrounded by the parietal tissue and naturally there can be no difficulty whatsoever in the matter of the supply of food material by this closely enveloping tissue to the embryo-sac. But in the "tenuinucellate" condition, the embryo-sac is left severely alone. The single layer of nucellus very soon perishes in the ontogeny of the ovule. There is a gap between the developing embryo-sac and the developing integument. There can be no question of a close contact of these two, such as exists between the parietal tissue and the embryo-sac in the Krassi-nucellate condition. Naturally, when contact, however imperfect, is established, between the developing embryo-sac and the integument, some sort of an intermediary tissue is found necessary in order to facilitate the free transference of nutritive material from the integument to the embryo-sac. Presumably, even this device is not sufficient, for in many cases, the endosperm haustorium makes its appearance as a post-fertilization structure. The tapetum after performing its function disintegrates, when the endosperm tissue is beginning to take up the nutritive role.

VI. Summary

The haploid chromosome number of *Vahlia oldenlandioides* has been confirmed to be 6, and that of *V. viscosa* has been determined to be 9.

The origin and development of the microsporangium, the embryo-sac, the endosperm and the embryo is described in detail; both *Vahlia viscosa* and *V. oldenlandioides* are found to be quite similar.

In both the species of *Vahlia* investigated, the endosperm is cellular and there are four uninucleate chalazal endosperm haustorial cells in both.

No relationship could be established between the division of the family on the basis of the type of endosperm development with (i) division of the family by taxonomists on morphological grounds and (ii) the chromosome numbers known in the family.

For a cytotaxonomical approach, cytological details like secondary association, etc., of which no information is now available, are suggested to be a necessary prerequisite.

The role of the integumentary tapetum is discussed in the light of its correlation to the nucellus and the endosperm.

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