STUDIES IN RUBIACEÆ

II. *Spermacoce hispida* Linn., *Guettarda speciosa* Linn. and Some Cytomorphological Considerations

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I. Introduction

PREVIOUS work in the family has been summarised by Raghavan and Rangaswamy (1941). The genus, *Spermacoce*, has not been worked out in detail by the previous workers, though the related genus *Diodia* has been studied to some extent.

Lloyd (1902) mentions that in *Spermacoce* the embryo-sac is slender and cylindrical. Fagerlind (1937) reports the presence of strophiole and a reduced type of nucellus. He classes the embryo-sac of *Spermacoce* under the *Richardsonia* type. None of the workers has investigated the genus *Guettarda*.

The present communication deals with the development of microsporangium, embryo-sac and embryo in *Spermacoce*. Some details of meiosis

in *Spermacoce* and *Guettarda* are described. The embryo-sac and the embryo development in *Guettarda* have also been described.

II. Materials and Methods

Materials of *Spermacoce* and *Guettarda* were available in plenty locally. For meiotic stages, the buds in the case of *Spermacoce*, and the anthers in the case of *Guettarda*, were fixed in Navaschin's fluid after prefixation in Carnoy's fluid. The required stages for fixation were determined through aceto-carmine examination.

Stages of microsporangium development and early stages of megasporogenesis for *Spermacoce*, were obtained from small buds fixed in formalin-acetic-alcohol. For the former, transverse sections were taken and for the latter, longitudinal sections were cut. For stages upto eight-nucleate embryo-sac, whole ovaries were fixed. For post-fertilization stages, ovules were taken out and fixed.

In the case of *Guettarda*, ovaries were fixed after removing the pubescent coating by means of a sharp blade. Because of the irregular orientation of ovules, many sections had to be cut before the required stages were obtained. Ovules were dissected out and fixed separately for post-fertilization stages.

The materials were dehydrated in alcohol and imbedded in paraffin wax using chloroform as the paraffin solvent. Sections were cut at thicknesses varying from 6 to 8 microns and stained in iron-alum hæmatoxylin and iodine gentian-violet.

III. Spermacoce hispida Linn.

(*a*) *Microsporangium development and Microsporogenesis.*—A transverse section of a very young anther shows at each of the four corners a hypodermal band of two primary archesporial initials (Figs. 1 and 2). Through the periclinal division of these cells, two layers of cells are formed, the outer being the primary wall layer and the inner, the primary sporogenous layer. The former divides and gives rise to more wall layers, while the latter gives rise to the sporogenous tissue (Fig. 3). In the present case, the sporogenous tissue is formed only to a limited extent. At the mature stage the wall of the anther sac consists of only three layers of cells (Fig. 4).

In addition to the three wall layers, a tapetal layer is differentiated from the innermost layer of the wall cells (Fig. 4). This consists of cells which during early stages are full of cytoplasm and are uninucleate (Fig. 5). Later they come to possess two nuclei (Fig. 6). This takes place by ordinary mitotic

division. Another interesting phenomenon is the fusion of two or more nuclei, resulting in a single nucleus with more than one nucleolus. Fig. 7 shows a binucleolated nucleus which is the result of fusion of two ordinary nuclei. Fig. 8 shows a tapetal cell, whose nucleus has three nucleoli, possibly from the fusion of a two nucleolated nucleus with an ordinary nucleus. Nuclei having more than three nucleoli were not found. Such a fusion of tapetal nuclei has been recorded in *Gynandropsis* (Raghavan, 1938) and in *Nicotiana glutinosa* (Raghavan and Srinivasan, A. R., 1941 *b*).

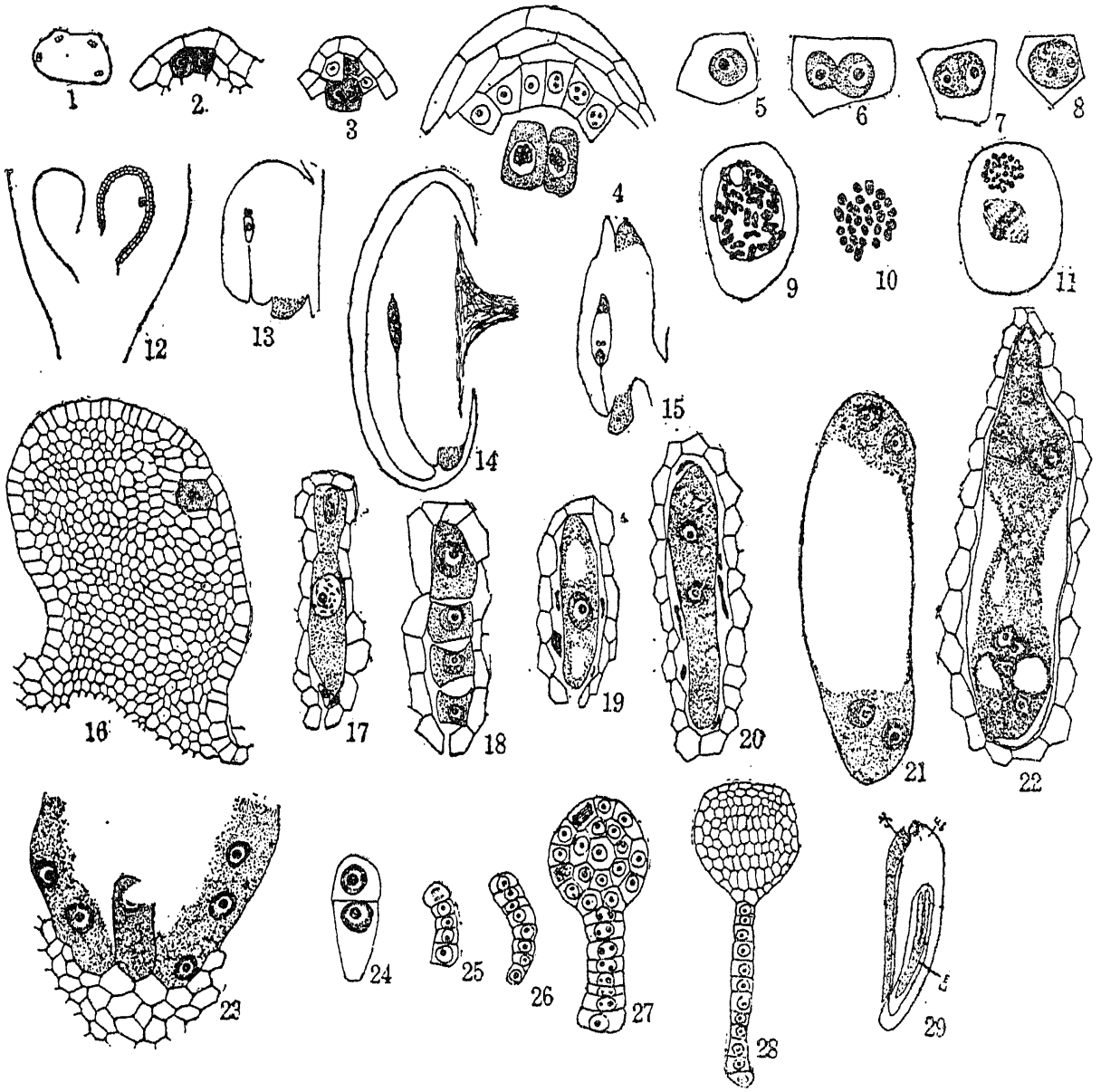
During prophase the pollen mother cells round themselves off and are very big in size. Owing to the slender nature of the chromatic threads and the comparatively large number, the details of synapsis before diakinesis could not be studied. At diakinesis 28 bivalents were counted, most of which were rod-shaped (Fig. 9). According to Nandi (1936) rod-shaped bivalents result from the small chromosomes, with one chiasma in one arm. Lawrence (1931) observes that the rod-shaped type is characteristic of small-chromosomed plants. The other bivalents are probably of the ring type.

Prometaphase follows diakinesis and the nucleus enters upon the first metaphase stage (Fig. 10). 28 Bivalents were counted of which three are somewhat bigger than the rest. Probably these three are the three ring bivalents observed during diakinesis: All the bivalents lie closely packed in the equatorial plate. This confirms the previously reported haploid chromosome number of the plant by Raghavan and Rangaswamy (1941).

The disjunction of bivalents is normal and at M II (Fig. 11). 28 Chromosomes could be counted at each pole of the P.M.C. The pollen grains are formed usually tetrahedrally. The pollen grains are comparatively large.

(*b*) *Development of embryo-sac and embryo.*—The ovary is two-carpelled and bilocular containing in each locule a solitary amphitropous ovule. The ovule at first arises as a straight protuberance from the base of the ovary (Fig. 12). The archesporium is differentiated laterally in a hypodermal position in the ovule. As the ovule grows and bends to assume the amphitropous configuration, the megaspore mother cell comes to point towards the base of the ovary. The ovule has a single massive integument (Fig. 13).

As the ovule bends to form the amphitropous structure, an outgrowth of the funicle is seen to develop on the side of the ovule towards the funicle (Figs. 13–15). This was termed the "strophiole" by Lloyd (1902) who found it in *Diodia*. It occurs in a number of other genera belonging to Coffeiodeæ such as *Richardsonia*, *Leptodermis*, *Pavetta*, *Ixora*, etc., and also in *Cephalanthes*, a genus belonging to Cinchonoideæ. A similar structure in



FIGS. 1–29. *Spermacoce hispida* Linn.

FIG. 1. Transverse section of a young anther. × 150. FIG. 2. Hypodermal band of two archesporial cells. × 750. FIG. 3. Formation of primary wall layer and primary sporogenous layer. × 750. FIG. 4. Mature anther with three wall layers, the tapetum and the pollen mother cells. The nuclei of the tapetum are multicleolated. × 750. FIG. 5. Uninucleate tapetal cell. × 1500. FIG. 6. A binucleate tapetal cell. Nuclei are in the process of fusion. × 1500. FIG. 7. A tapetal cell with a nucleus which has two nucleoli. × 1200. FIG. 8. Tapetal cell whose nucleus has three nucleoli. × 1200. FIG. 9. Diakinesis in P.M.C. × 2200. FIG. 10. First metaphase. × 2200. FIG. 11. Second metaphase. × 2200. FIG. 12. Longitudinal section of an young ovary showing the origin of the ovules. × 500. FIG. 13. Ovule showing the megaspore mother cell, integument, micropylar canal and the strophiole. Strophiole is represented by the dotted portion. × 150. FIG. 14. Ovule at the time of binucleate embryo-sac stage. × 75. FIG. 16. Archesporium of the megaspore. × 500. FIG. 17. Megaspore mother cell. × 750. FIG. 18. Linear tetrad. × 750. FIGS. 19–21. Uni-, bi-, four-nucleate embryo-sacs. × 750. FIG. 22. Mature embryo-sac. × 500. FIG. 23. Zygote and nuclear endosperm. × 500. FIG. 24. Two-celled proembryo. × 500. FIGS. 25 & 26. Linear proembryos of 5 and 8 cells. × 150. FIGS. 27 & 28. Mature embryos. × 350; FIG. 29. Section of a mature seed. × 10. *In.*, Integument; *Em.*, Embryo; *St.*, Strophiole.

Coffea has been termed as *obturator* by Howk (1938). Whatever its name may be, its function is not definitely known. Lloyd (1902) attributes to it a nutritive function and thinks that "it may be the seat of some metabolic activity during the growth of the embryo and the endosperm".

From the position it occupies (Fig. 15) there is much room to think that it controls in some way the ingress of the pollen-tube into the micropylar canal. But this can be substantiated only after critical pollination and fertilization studies.

The archesporium is enveloped by two or three cells which form the nucellar epidermis. As the megaspore mother cell enlarges, the two nucellar cells are crushed, caught as they are between the massive integument and the enlarged megaspore mother cell (Fig. 17). The exact type of nucellus in the present case could not be made out. But the figures of Fagerlind (1937) show very well that a straight row of cells cover the archesporium upto the linear tetrad stages in *Spermacoce tenuior*, and he regards it as belonging to the *Vaillantia*-type.

The archesporium is differentiated from the hypodermal layer. It consists of a single cell. The cells near the archesporium are somewhat elongated (Fig. 16). Fig. 17 represents a megaspore mother cell and another cell towards the chalazal end. This is also shown in Fig. 13, as occurring just below the megaspore mother cell. Presumably this is an additional megaspore mother cell situated below the normal one which, after some time, is arrested in its development and functions as any other ordinary cell of the surrounding integument. Multicellular archesporium is not rare in this family. Though not common in this genus, other genera like *Galium*, *Callipeltis*, etc., have been known to exhibit two or more megaspore mother cells, each of which was found to develop into the linear tetrad in the normal manner (Lloyd, 1902).

The megaspore mother cell gives rise to the linear tetrad of megaspores (Fig. 18). The chalazal megaspore grows into the uninucleate embryo-sac (Fig. 19). The uninucleate embryo-sac develops into the mature embryo-sac according to the monosporic type of embryo-sac development (Maheshwari, 1937) (Figs. 20–22).

The growth of the embryo-sac is very rapid and the size of the mature embryo-sac is very large (Fig. 22). The latter is cylindrical, tapering at the chalazal end. The egg apparatus consists of well-defined synergids and egg cell. The polar nuclei which are about to fuse lie closely adpressed to the egg cell. The most remarkable feature is the nature of the antipodals. The antipodals are very large with prominent nuclei. The lowermost of the three

antipodal cells has a tapering end and is the biggest. The antipodal apparatus bears a great resemblance to the early stages in the development of antipodal haustonia which occur in many genera of this family (*e.g.*, *Callipeltis*, *Asperula*, *Diodia*, etc.) (Lloyd, 1902). In this case however, they degenerate after fertilization.

The fertilized egg divides transversely and develops into the proembryo (Fig. 24). The endosperm nucleus divides by free nuclear division and gives rise to nuclear endosperm (Fig. 23). Cell wall formation in endosperm tissue takes place later.

Through repeated divisions, an eight-celled linear proembryo is formed (Figs. 25 & 26). Further development appears to be normal. One important feature of this species is the unusual length of the suspensor which is made up of 12 cells at the mature stage. The suspensor is uniseriate (Figs. 27 & 28). Such a long uniseriate suspensor is met with in *Leptodermis* (Fagerlind, 1937) and in *Diodia* (Lloyd, 1902).

The mature seed (Fig. 29) consists of the remains of the strophiole (*St*) and the thick integument (*In*), the latter of which does not perish as in *Dentelal* and *Oldenlandia*. The cells of the integument become thick-walled and the seed thus is very hard ultimately. There is no endosperm surrounding the embryo (*Em*).

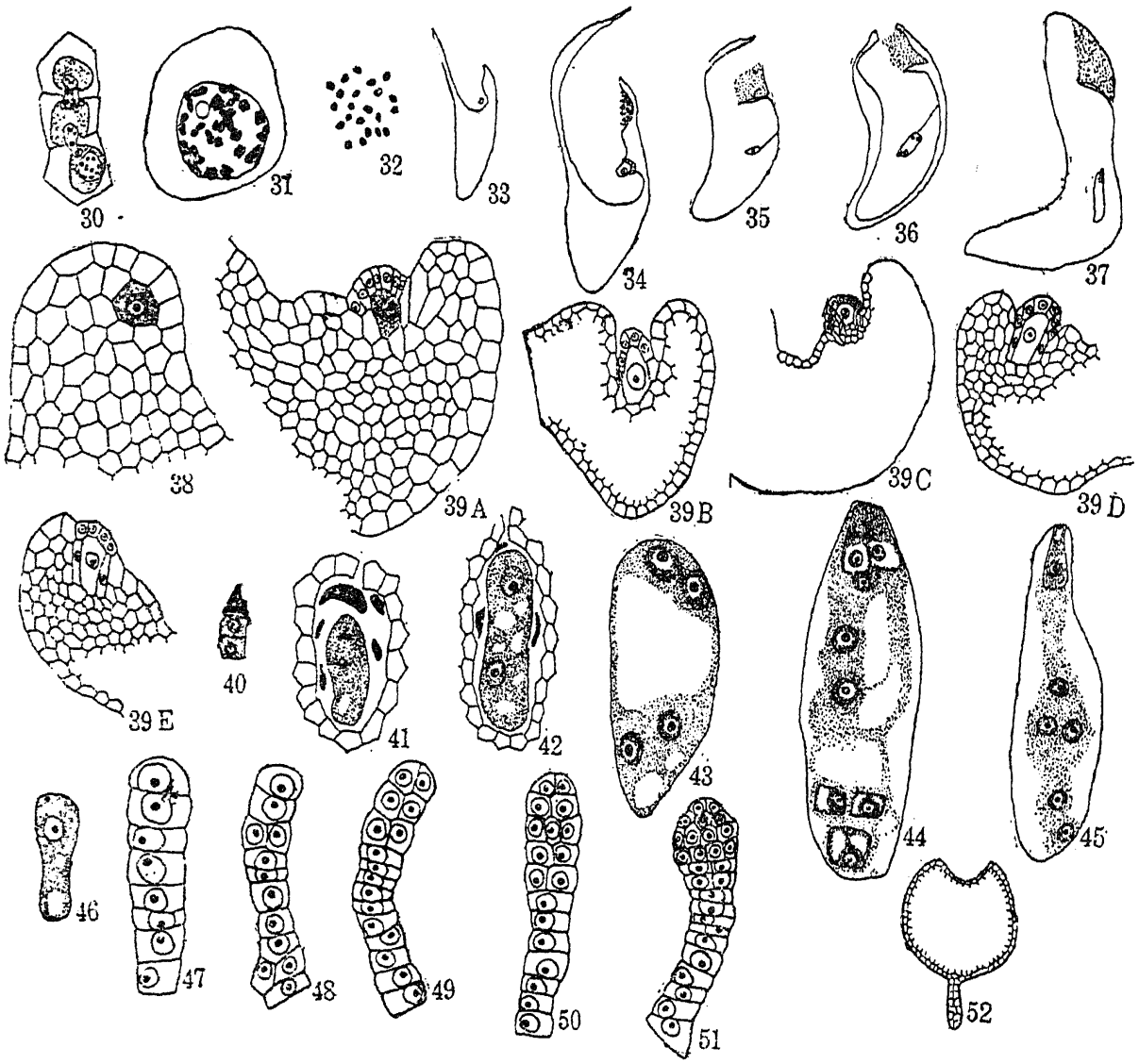
IV. Guettarda speciosa Linn.

(*a*) *Microsporogenesis.*—The prophase studies in the P.M.C. of *Guettarda* occasionally show the interesting phenomenon of cytomyxis (Fig. 30). Cells exude their nuclear contents into adjacent cells. Such a feature has been found characteristic of interspecific hybrids and has been observed in the case of the hybrid between *Nicotiana tabacum* × *N. glutinosa* (Raghavan and Srinivasan, A. R., 1941*a*.) It has also been found in *Tridax procumbens* (Raghavan and Venkatasubban, 1941). Among the three cells, in the first cell, extrusion takes place through three passages and in the other cell through a single passage. The phenomenon occurs only rarely and does not affect the meiotic regularity of the plant.

During diakinesis, 22 bivalents are observed. The rod and the ring types of bivalents are found (Fig. 31).

During metaphase, the 22 bivalents arrange themselves in the equatorial plate. They do not lie compactly as in the case of *Spermacoce*, but lie dispersed in the equatorial plate.

The rest of the meiosis takes place normally with the production of good viable pollen.



FIGS. 30–52. *Guettarda speciosa* (except Figs. 39 B, C, D and E)

FIG. 30. Cytomyxis in pollen mother cells. × 700. FIG. 31. Diakinesis. × 2200. FIG. 32. First metaphase. × 2200. FIG. 33. Ovule and ovarian chamber, when archesporium is just differentiated. × 150. FIG. 34. Ovule and ovarian chamber when integument is differentiated. × 150. FIG. 35. Ovule at the time of uni-nucleate embryo-sac. × 75. FIG. 36. Ovule during four-nucleate embryo-sac. × 75. FIG. 37. Mature ovule, in which a mature embryo is formed. × 10. Strophiole shown stippled in Figs. 34–37. FIG. 38. Archesporium. × 750. FIG. 39 A. Showing nucellus, archesporium and integument. × 750. FIG. 39 B. Ovule of *Ophiorrhiza* showing a convex layer of nucellus consisting of five cells. × 750. FIG. 39 C. Nucellus of *Canthium*. × 355. FIG. 39 D. Nucellus occurring in *Mussaenda*. × 750. FIG. 39 E. The nucellar epidermis of *Pentas*. × 750. FIG. 40. Linear tetrad with the two micropylar cells degenerated. × 750. FIGS. 41–43. One-, two-, and four-nucleate embryo-sacs. × 750. FIG. 44. Mature embryo-sac. × 750. FIG. 45. Oospore and endosperm nuclei. × 500. FIG. 46. Oospore. × 750. FIGS. 47–52. Embryo stages. × 750. FIG. 52. Lobing of cotyledons in the embryo. × 150.

(*b*) *Embryo-sac and embryo development.*—The ovary is syncarpous consisting of 7 to 9 carpels and as many locules. The ovules are solitary and pendulous. They first arise as pendulous protuberances in the carpel chamber (Fig. 33). The archesporium is differentiated on the free end of the

ovule and points downwards. As the ovule grows, it curves along its long axis and the archesporium comes to assume a horizontal configuration (Fig. 34). The single integument is now differentiated and it grows into a massive structure, so that the archesporium comes to lie deep in the ovule. The micropylar canal is formed. Still later the ovule curves more, causing the embryo-sac and the micropylar canal to form an acute angle with the long axis of the ovule (Figs. 35 & 36). After the four-nucleate embryo-sac stage, the part of the ovule at the antipodal end elongates and curves away from the side in which the embryo-sac is situated. The result is that the mature ovule is not straight, but is bent. The embryo-sac comes to be situated during this stage, just at the portion of the bend (Fig. 37).

The funicle is thick. The growth of the integument results in the formation of the micropylar canal. When the integument is differentiated, the strophiole develops as an outgrowth from the funicle (Fig. 34). Its growth is soon arrested and it is seen in later stages as an outgrowth of the funicle capping the ovule at its basal portion. The strophiole in this case is not so very prominent as in *Spermacoce* (Figs. 35, 36 & 37). It could be referred to as merely the funcile but for the fact that it is not connected to the rest of the ovular body as the funicle should be.

The archesporium differentiated from the hypodermal layer, consists of a large single cell with a prominent nucleus (Fig. 38). At later stages the integument is differentiated (Fig. 39 A). With the growth of the integument, the archesporium comes to occupy a deep position in the ovule. There it elongates into the megaspore mother cell, which undergoes meiosis and results in the linear tetrad. Of the tetrads, the two micropylar cells degenerate soon, and the third degenerates only slowly. The chalazal megaspore develops (Fig. 40).

When the archesporium is differentiated and the integument arises, the former is covered by seven cells which form the somewhat convex nucellar epiderms (Fig. 39 A). This bears a great resemblance to the nucellus of *Phyllis*. So the nucellus in the present case belongs to the primitive *Phyllis*-type. With the development of the integument, the nucellar layer is crushed.

Due to the growth of the megaspore into uninucleate embryo-sac, the integumental cells in the neighbourhood of the embryo-sac disintegrate (Fig. 41). The nucleus of the uninucleate embryo-sac divides and the daughter nuclei travel to opposite poles. Fig. 42 shows the two-nucleate embryo-sac. The rapid growth in size of the embryo-sac results in the further degeneration of the immediately surrounding integumental cells (Fig. 42). By further division of the nuclei the four- and eight-nucleate embryo-sacs are

formed (Figs. 43 & 44). The mature embryo-sac has the synergids and the egg cell at the micropylar end. At the chalazal end there are three antipodals. The antipodals are of normal size and all the three are of the same size unlike those of *Spermacoce*. The polar nuclei are found in the centre of the embryo-sac (Fig. 44) and later fuse to give the secondary nucleus.

After fertilization, the endosperm nucleus divides first and gives rise to the nuclear endosperm (Fig. 45). The oospore divides repeatedly until a linear, uniseriate eight-celled proembryo is formed (Fig. 47). The cells towards the embryonal end undergo anticlinial divisions and become biseriate (Figs. 48 & 49). The embryo proper appears to be developed from the three or four apical layers (Figs. 50 & 51). The portion of the suspensor immediately next to the embryo proper is bi-seriate. Multiseriate suspensors occur in other genera of Rubiaceæ, where the cells elongate and perform haustorial functions, for example, *Asperula*, *Vaillantia*, *Galium*, etc.

The mature seed is enclosed in the locule of the ovary whose wall becomes much thickened.

V. Discussion

(*a*) *Some cyto-morphological considerations.*—In a previous paper, Raghavan and Rangaswamy (1941) have discussed the basic chromosome number of the family Rubiaceæ and have suggested 11 as the possible number. In *Oldenlandia* and *Dentella* a rudimentary (single-celled) nucellus was met with. This advanced feature was correlated to the chromosome number being in the nine series. It was therefore suggested that 9 could not be the basic number, but some other number higher than that. The most frequently occurring number was 11 and this was exhibited by members possessing the so-called primitive type of nucellus, *i.e.*, the *Phyllis*-type of nucellus with seven to eight nucellar cells forming a convex layer capping the archesporial cell. The other numbers were suggested to have arisen secondarily by various means.

The present morphological study of these two genera seems to corroborate the previous findings. Some interesting observations suggest themselves on a closer scrutiny of the chromosome numbers known so far, as correlated to this important morphological feature, *viz.*, the nature of the nucellar epidermis. Based chiefly upon this factor, 11 was suggested to be the possible basic number of the family. Members exhibiting this number are characterised by the so-called *Phyllis*-type of primitive nucellar epidermis, composed of 6 or 7 cells forming a convex layer. The other members derived therefrom must be regarded as secondary numbers and as such the genera exhibiting these numbers must show a type of nucellar epidermis which must be regarded as more

advanced than the *Phyllis*-type. *Prima facie* these two genera investigated in this paper, *Spermacoce* and *Guettarda*, corroborate these inferences. *Guettarda* which is in this investigation reported to have $n = 22$ (belonging to the 11 series) must, if the previous suggestion was correct, show the *Phyllis*-type of nucellar epidermis (Fig. 39 A). And this is just what we find. To secure further corroboration for this, we have studied the nucellar epidermis in other genera showing the 11 number. The genera that we have studied and figured are *Ophiorrhiza*, *Canthium* and *Mussaenda* (Figs. 39 B, 39 C & 39 D respectively). These show definitely the *Phyllis*-type of nucellus. *Pentas*, which is another species investigated (Fig. 39 E), shows $n=10$ and in this, the nucellus is not of the *Phyllis*-type, but of the *Bouvardia*-type, *i.e.*, two to four cells forming a straight row.

We have made a critical study of the data presented in Fagerlind's paper and endeavoured to find out, if the evolution of the nucellar epidermis such as is indicated by Fagerlind, could in any way throw light on the evolution of the chromosome numbers in the family. According to Fagerlind, who does not use chromosome numbers to support his views upon nucellar evolution, the *Phyllis*-type is the most primitive. On a close examination of the different genera comprised under this group, the vast majority belong to the 11 series. The only exception would appear to be *Hoffmannia* showing $n = 12$. The genera coming under this group are as follows:—*Phyllis*, *Psychotria*, *Cephalanthus*, *Chiococca* (all belong to the 11 series) and *Hoffmannia* ($n = 12$) (Fagerlind), *Ophiorrhiza*, *Canthium*, *Guettarda* and *Mussaenda* (all belong to 11 series) (Authors).

From this *Phyllis*-type the *Bouvardia*-type is derived, wherein the nucellus is straightened with about four cells. Plants showing this type of nucellus are *Bouvardia* ($n = 9$) and *Pentas* ($n = 10$). From this *Bouvardia*-type, according to Fagerlind, evolution has proceeded along two lines, one resulting in the *Oldenlandia*-type with only one nucellar cell. Along this line reduction has presumably taken place from a four-celled condition to an one-celled condition as in *Oldenlandia* and *Dentella*. A still further reduction along this line leads to the complete suppression of the nucellar cells, *e.g.*, *Houstonia*. Along the other line evolution is suggested to have taken place in the direction of increased nucellar cells culminating in the so-called *Vaillantia*-type. Plants showing this type of nucellus are *Mericarpea* ($n = 11$), *Galium* ($n = 10$ and 11), *Callipeltis* ($n = 11$), *Sherardia* ($n = 11$), *Rubia* ($n = 11$), *Putoria* ($n = 11$), *Warburgina* ($n = 11$), *Asperula* ($n = 10$ and 11), *Phuopsis* ($n = 10$ and 11), *Vaillantia* ($n = 9$), *Spermacoce* ($n = 14$) and *Richardsonia* ($n = 14$) (Fagerlind). Whereas in *Bouvardia*-type there is only a straight row of two to four cells,

there are about six to seven cells in the *Vaillantia*-type. This scheme of nucellar evolution is shown diagrammatically in Diagram A.

Trying to correlate the chromosome numbers known so far, with this line of evolution as suggested on the basis of the nucellar epidermis alone, we find that, though in the main it is corroborative, yet there are a few difficulties. If it is conceded that 11 is the primitive number—for this there seems to be ample evidence now at hand—then, the difficulty would arise in respect of the *Vaillantia*-type. This, according to Fagerlind, possesses an advanced type of nucellus with a straightened row of seven cells, having been derived from the *Bouvardia*-type. If this is so, the majority of the plants belonging to this type must show a number other than 11, either more or less. On the other hand, we find that the majority of the genera under this type would appear to possess 11. *Vaillantia* itself shows only 9. There are others which show numbers higher than 11, *e.g.*, *Spermacoce* and *Richardsonia*. Except *Vaillantia* all the others show either 11 or generally more than 11. It would therefore appear to us more reasonable to derive this *Vaillantia*-type directly from the *Phyllis*-type, without the intervention of the *Bouvardia* stage. Cytologically it would mean the evolution from a eleven-chromosomed *Phyllis* ancestor, of genera showing chromosome numbers of 11 and more, exhibiting the straightened type of nucellus. We venture to believe that the evolution of a straightened seven-celled nucellus from a convex seven-celled nucellus would be more natural, than its evolution from a *Bouvardia*-type. The latter method would mean that, first of all, from a convex seven-celled nucellus the *Bouvardia*-type was derived involving not only a straightening, but also a reduction in the number of cells. This reduced number has to increase again in order to attain the *Vaillantia* condition. This is rather strained and we believe that the evolution of the *Vaillantia*-type directly from *Phyllis* would be more natural. The chromosome numbers also seem to support this view.

On the other side, from the *Phyllis*-type, the *Bouvardia*-type may be derived. By further reduction the *Oldenlandia*-type is got. Still further reduction leads to the *Houstonia*-type. On the basis of chromosome numbers such an evolution would appear to be supported. All these types show chromosome numbers less than 11. *Bouvardia*-type includes genera showing 9 and 10. *Oldenlandia* and *Houstonia*-types include genera belonging to the nine series only. This scheme of evolution is represented diagrammatically in Diagram B.

Evolution presumably has proceeded along two divergent lines from the *Phyllis*-type. Along one line the number of nucellar cells has remained

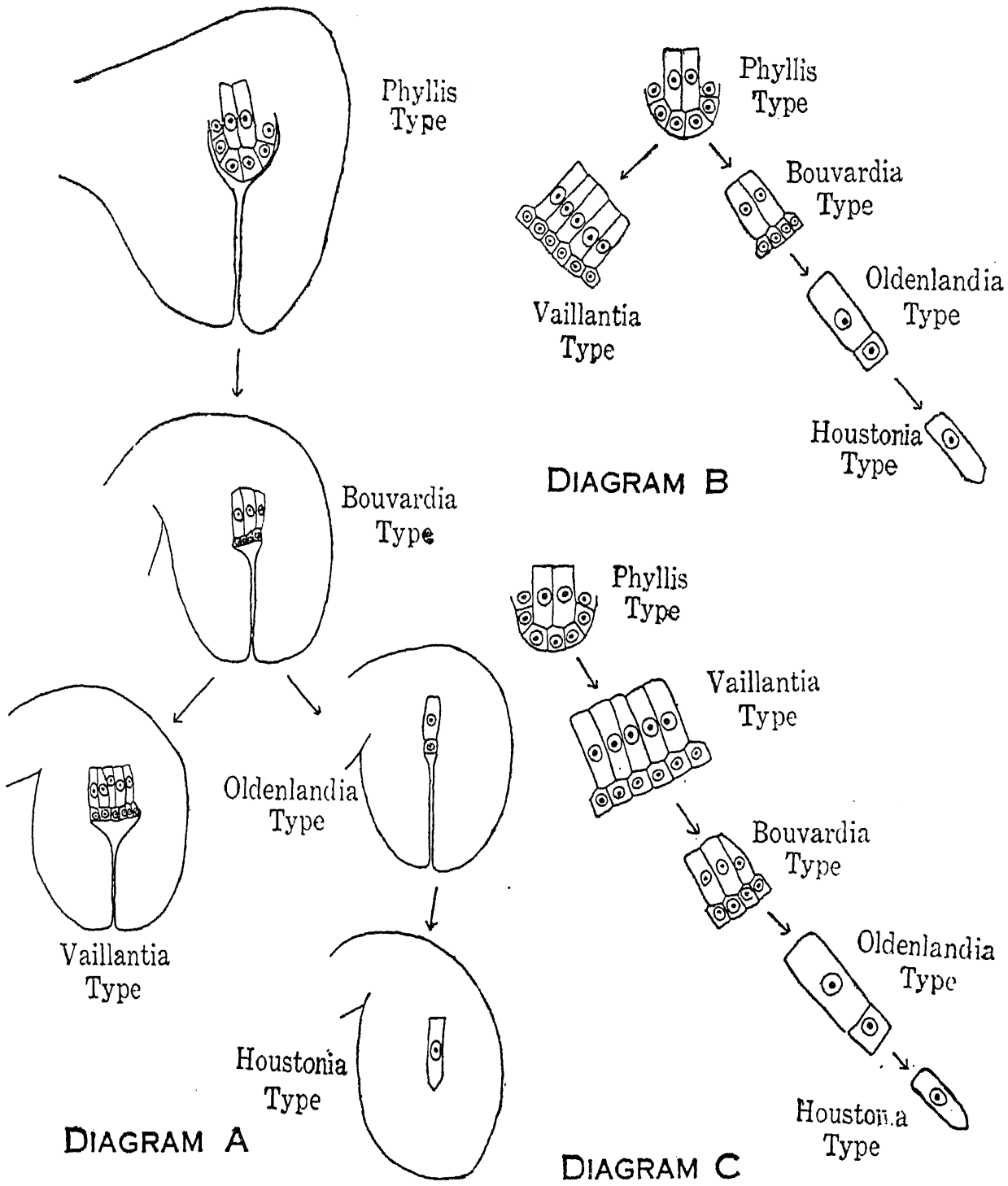


DIAGRAM A. Showing the evolution of the nucellus in the Rubiaceæ according to Fagerlind's concept.

DIAGRAMS B & C. Illustrating alternative schemes of nucellar evolution suggested in the present paper. Explanation in the text.

constant, only they have straightened out. In these, chromosome numbers have also remained unchanged in the vast majority of cases and such of the deviations as are met with like *Spermacoce* and *Richardsonia*, are on the side of increased numbers. Along the other line, nucellar reduction would appear to be correlated with reduction in chromosome numbers, all these belonging to the nine series.

There is yet another possibility that should also be considered, *viz.*, the straight evolution along a single line from the *Phyllis*-type instead of their being two divergent lines of evolution. From the *Phyllis*-type we get the *Vaillantia*-type (straightened, but same number of cells) from *Vaillantia*, the *Bouvardia*-type (straightened, but number of cells reduced), from this the *Oldenlandia*-type (still further reduction to single cell) and ultimately to the *Houstonia*-type (nucellus completely suppressed). This scheme has its merits also. Primarily the stray occurrence of numbers less than eleven in the *Vaillantia*-type (*Vaillantia*—9, *Phuopsis*—10, *Asperula*—10), may be explained, *i.e.*, from the *Phyllis*-type to the *Vaillantia*-type there is no change in the number of nucellar cells, only there is a straightening. The majority of the plants remain unchanged in chromosome numbers also, but, even in this group, we already see a tendency towards a reduction in the chromosome numbers from 11 to 10 and 9. This becomes a constant feature in the succeeding types like *Bouvardia*, *Oldenlandia* and *Houstonia*. Only, in these groups this reduction of chromosome number is accompanied by a reduction in the nucellar epidermis. In *Bouvardia*, with only four cells, we get nine and ten. In *Oldenlandia*- and *Houstonia*-types, we get only nine seried plants so that, the ten and nine plants, the few that there are under the *Vaillantia*-type, must be regarded as representing a tendency for reduced chromosome numbers unaccompanied by a reduction in the nucellar cells. This however soon follows and the result is the *Bouvardia*-type from which the others arise. This is represented schematically in Diagram C.

It must also be admitted at the same time that in the very *Vaillantia*-type, there are some genera showing numbers more than 11, *Spermacoce* and *Richardsonia*. What these portend we do not know, primarily because we have no morphological evidence for plants showing numbers higher than 14, etc., for *e.g.*, for *Posoqueria* belonging to the 17 series we have no information about the nature of nucellus.

We have comparatively enough data for plants exhibiting the numbers less than 11. All these, whether belonging to *Bouvardia*- or the *Oldenlandia*-type, show reduced nucellus either four or one-celled. Their number is most generally 9, ten being shown by very few only. Since these numbers occur occasionally in the *Vaillantia*-type, we have suggested that these reduced numbers, unaccompanied by reduced nucellus, already portend the reduced nucellus, that becomes the constant feature in the *Bouvardia* and *Oldelandia* types. In the same way, what the stray occurrence of increased numbers like 17, etc. are suggestive of, we can only say if we had some data pertaining to the nature of nucellus in plants showing the higher numbers. We know that

the 14-seried plants like *Spermacoce* show the *Vaillantia*-type of nucellus. In other words, just as a stray decrease of chromosome numbers amongst this type unaccompanied by any nucellar reduction, has been held to exhibit the tendency for nucellar reduction, such as is exhibited by the *Bouvardia* and *Oldenlandia* types, so, the increased numbers must be regarded as indicating a tendency towards increased nucellar cells. If we had details for any higher chromosomed genera, then, this anticipation could be verified even as in the case of the reduced numbers correlated to reduced nucellar cells. But, pending this, the suggestion can only be made that the increased numbers must either show the *Vaillantia*-type of nucellus or a nucellus with more cells than in the *Vaillantia*-type. Whether this anticipation is correct, future investigations alone can tell.

(*b*) *Strophiole*; *has it any phylogenetic significance ?*—In Rubiaceæ, it is difficult to find a morphological character of strict phylogenetic significance except the nucellar epidermis. The strophiole is present in most of the genera irrespective of whether the genera are primitive or advanced, determined from apt considerations like the nature of nucellus. Thus for example, it is present in *Guettarda* which has a primitive nucellus and is at the same time present in *Spermacoce* which has got an advanced type of nucellus. Moreover, it does not characterise genera which belong to a particular chromosome type. It does not even characterise the genera belonging to tribes of ordinary classification, based on the number of ovules in the carpel. It is thus present both in Coffeoideæ and Cinchonoideæ, though only rarely in the latter. So the presence of strophiole can be dismissed as possessing little phylogenetic significance.

VI. Summary

The development of embryo-sac and embryo has been described in *Spermacoce hispida* and *Guettarda speciosa.*

The haploid numbers, *Spermacoce* ($n = 28$) and *Guettarda* ($n = 22$), previously reported, are confirmed. Meiosis is described in some detail.

The development of the strophiole has been traced and it is considered to have little phylogenetic importance.

The suggestion previously made that 11 was the basic number of the family is confirmed by data gathered in this paper.

The evolution of the chromosome numbers in this family is discussed in the light of data known in connection with nucellar evolution. A correlation is established between the two and a few tentative schemes of nucellar and chromosomal evolution suggested.

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