THE DEVELOPMENT OF THE FEMALE GAMETOPHYTE IN SOME MEMBERS OF THE EUPHORBIACEÆ

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ALTHOUGH in recent years a considerable amount of embryological work has been done in India and abroad, the family Euphorbiaceæ has not, however, received the amount of attention it deserves, considering the large number of species it includes. Schnarf[12] has reviewed the relevant literature on the subject upto the year 1931. According to him the general mode of development of the female gemetophyte is of the normal type. A few abnormal types have been recorded and classified under different heads such as Phyllanthoideæ, Crotonoideæ, etc. Later work has been recently reviewed by Maheshwari.[8]

Not much work has been done in India on this family. Maheshwari and Chowdry[7] published a short note on the development of the embryo-sac of *Phyllanthus niruri* collected from Agra. The mature embryo-sac appeared to be 4-nucleate due to the degeneration of the antipodals. Later Maheshwari and Johri[9] published a short account of the development of the female gematophyte in *Acalypha indica* and reported the occurrence of a tetraporic 16-nucleate embryo-sac of the "Penæa form" under the Peperomia type. The organization of the mature embryo-sac shows a great variation. Miss Bhalla[2,3] studied the development of the female gametophyte in *Euphorbia helioscopia* and *Euphorbia Royleana*. In the former the archesporial cell is hypodermal in origin and cuts off an upper parietal cell and a lower megaspore mother cell. The latter gets buried deeply into the nucellus by the activity of the parietal cell. In *Euphorbia Royleana* she noticed sterility due to the non-development of the embryo-sac beyond the archesporial stage. The archesporial cell, however, is found buried 2–3 cells deep in the nucellus.

Very recently Maheshwari[6] has studied the development of the embryo-sac of *Euphorbia heterophylla*. This had been first investigated by Modilewski[10] who reported it to be of the Normal-type. Sanchez[11]

re-investigated it and found the embryo-sac to be tetraporic and 8-nucleate (*Adoxa type*). Maheshwari, however, reports a Normal-type of development. Kajale and Rao[4] have very recently described the development of the female gametophyte in *Euphorbia hirta* and *Jatropha gossypifolia*. They found the presence of an obturator and a normal type of development of the embryo-sac in both the plants.

Material and Methods

The following plants furnished the material on which the investigation is based :—

1. *Putranjiva Roxburghii* Wall.
2. *Trewia nudiflora* Linn.
3. *Phyllanthus niruri* Linn., and
4. *Euphorbia thymifolia* Burm.

Of these the first two are dicœcious and are road-side trees commonly growing in Calcutta, while *Phyllanthus niruri* and *Euphoribia thymifolia* are small monœcious herbs. The former commonly occurs in waste fields, while the latter is found generally on the walls of old buildings.

Young ovaries and flower bunds at different stages of development were fixed in the field generally between 8–11 A.M., and between 1–3 P.M. In advanced stages, the ovaries of *Putranjiva Roxburghii* and *Trewia nudiflora* become greatly enlarged and hence to facilitate quick and easy penetration of the fixing fluids, the superficial and unnecessary tissues of the ovarian walls were trimmed off carefully without injuring the ovules.

Allen's modified Bouin's and Nawaschin's fluids were used for fixation. Both gave fairly good results but Nawaschin's fluid appeared to give slightly better results. In the case of *Euphorbia thymifolia* difficulties were experienced in fixing, possibly due to the presence of excessive latex in its tissues. The materials after fixation were dehydrated and cleared in the usual way, and finally imbedded in paraffin.

Sections were cut from 6–16 microns thick, the thickness depending on the stages of development required for study. Heidenhain's Iron-Alum Hæmatoxylin was used for staining.

Observations

(*a*) *The Integuments*

The ovules are anatropous and bitegmic. In *Putranjiva Roxburghii* and *Trewia nudiflora* both the integuments in the mature ovule take part in the formation of the micropyle which is fairly long. In *Phyllanthus*

niruri and *Euphorbia thymifolia,* however, no true micropyle is formed. In the former, at the mature embryo-sac stage, the nucellar beak remains protruded beyond the integuments (Text-fig. 8), while in *Euphorbia thymifolia* the nucellus and the integuments lie almost at the same level (Text-Fig. 2).

The thickness of the integuments varies in the different plants, depending on the size of the ovules, large ovules having thicker integuments. Thus in *Putranjiva Roxburghii* and *Trewia nudiflora* the integuments were 3 to 9 cell layered, while in *Phyllanthus niruri* and *Euphorbia thymifolia* they were 2 to 4 layered. In *Trewia nudiflora* the outer integument becomes so massive that at first sight the ovules appear to be covered by a single integument.

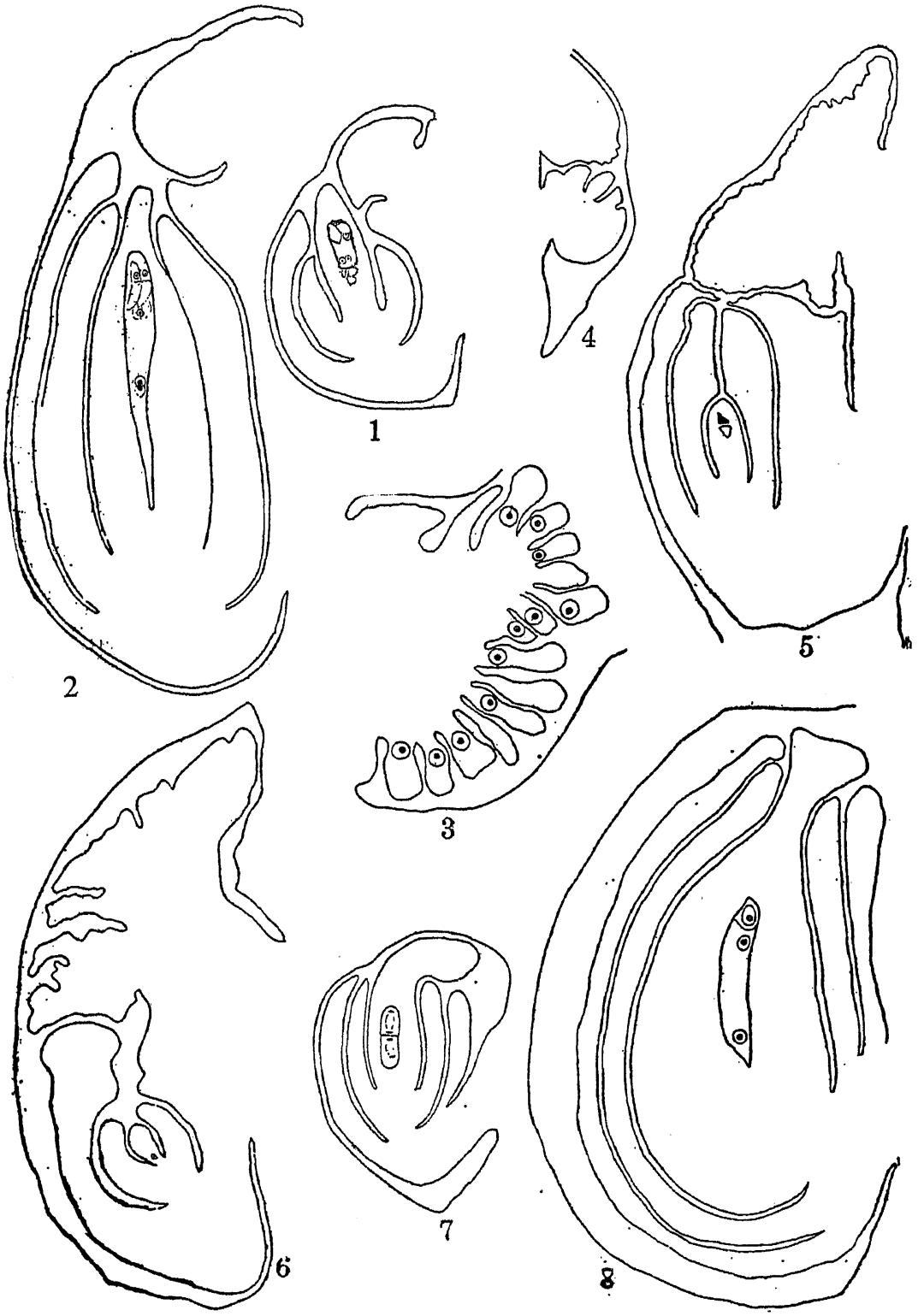
(*b*) *The Nucellus*

In *Putranjiva Roxburghii* and *Trewia nudiflora* the nucellus is comparatively massive and the megaspore mother cell is deep seated. At a very early stage of the development of the female gametophyte the nucellus appears to be completely encased by its integuments.

Phyllanthus niruri and *Euphorbia thymifolia* are characterized by the presence of nucellar beaks. This has been noted previously in the former plant by Maheshwari and Chowdry[7] in 1937. Miss Lyon[5] has noted the presence of a nucellar beak in *Euphorbia corollata.*

The " nucellar beak " as observed in the course of the present study shows a difference in its development and orientation. In *Phyllanthus niruri* in the megaspore mother cell stage, the nucellus protrudes out and the integuments just make their appearance as is commonly seen in other plants. Along with the growth of the integuments the nucellus also undergoes marked development and just prior to the reduction division of the megaspore mother cell, the two integuments and the nucellus stand almost at the same level. From this stage onwards, particularly during the second division of the megaspore mother cell the growth of the nucellus appears to be comparatively rapid and it protrudes out in the form of a beak which very soon comes in contact with the ovarian wall and curves towards the placenta as shown in Fig. 7. In post-fertilization stages a further growth of the integuments takes place and they lie at the same level as the tip of the nucellar beak. (Text-Fig. 8).

In *Euphorbia thymifolia,* protrusion of the nucellar tip becomes evident at t e 2-nucleate stage of the embryo-sac. It does not curve but retains its normal orientation even in the mature ovule, where it is finally enveloped by the integuments, leaving a wide micropyle through which the nucellar beak protrudes slightly (Text-Fig. 2). In *Euphorbia hirta* Kajale and Rao[4] found the nucellar beak to protrude in the form of a long finger-like process.



Text-Figs. 1-8. *Euphorbia thymifolia.*—Figs. 1 and 2. Stages in the development of the integuments and the obturator. × 450. Fig. 3. The hair-like uninucleate processes of the obturator. × 1540. *Putranjiva Roxburghii.* Figs. 4 and 5. The development of the obturator. ×155. *Trewia nudiflora.* Fig. 6. The position of the integuments and the obturator at the M. M. C. stage. × 155. *Phyllanthus niruri.* Fig. 7. The position of the integuments and the nucellar beak during the 2nd division of the M. M. C. × 450. Fig. 8. The position of the integuments and their nucellar beak at the mature E. S. and post-fertilization stages. × 450.

(*c*) *The Obturator*

The presence of an obturator has been recorded in some genera only, such as *Euphorbia, Croton* and *Acalypha.* In the present study it was observed that an obturator is present in *Putranjiva Roxburghii, Trewia nudiflora* and *Euphorbia thymifolia.* Except in the last, the obturator is fairly well developed even at the megaspore mother cell stage (Text-Figs. 4 and 6). In *Euphorbia thymifolia,* however, only a slight protrusion from the placenta occurs at this stage. The later development of the obturator shows no marked difference. It results in the formation of a hump-like structure which lies above the micropyle (Text-Figs. 1 and 2). It is interesting to note, however, that in *Euphorbia thymifolia* the outermost cells composing the obturator grow out in the form of hair-like processes and are uni-nucleate (Text-Fig. 3). Kajale and Rao[4] have noted the presence of an obturator of the loose type in the species studied by them. On *Putranjiva Roxburghii* and *Trewia nudiflora* the obturator becomes very massive during the later stages of the development of the female gametophyte and comes to lie just above the micropyle, so that the pollen tube has to follow a circuitous route to reach the embryo-sac (Text-Figs. 5 and 6).

Maheshwari and Chowdry[7] report the presence of an obturator in *Phyllanthus niruri.* The present study, however, failed to reveal its presence. It is probable that they mistook the nucellar beak of the second ovule for an obturator. In this connection, some preparations of *Phyllanthus urinaria* were also examined and here too the absence of an obturator was noted. There were two ovules in each loculus and the nucellar beak was very prominent. In longitudinal sections, the nucellar beak of the second ovule sometimes gave the appearance of an obturator but close examination always revealed its true nature.

(*d*) *The Development of the Macrospores*

The presence of a multicellular archesporium has been noted previously in various species of *Euphorbia.* The present study, however, shows that in *Euphorbia thymifolia* a single archesporial cell occurs in the hypodermal layer of the nucellus and by its division gives rise to the megaspore mother cell and a cover cell (Text-Fig. 9). In *Putranjiva Roxburghii* a multicellular archesporium has been observed from which a single megaspore mother cell develops and is first noted in the 4th layer of the nucellus (Text-Fig. 10), while in *Trewia nudiflora* it generally occurs in the 11th layer and in *Phyllanthus niruri* in the 3rd layer. It might be, as Maheshwari and Chowdry[7] suggest, that the archesporial cell cuts off a parietal layer and then functions as the megaspore mother cell. The megaspore mother cell of *Phyllanthus*

niruri in the later stages of its development is pushed considerably inside the nucellus on account of the rapid divisions of the overlying cells and comes to lie in the 9th or 10th layer.

The megaspore mother cell increases in size before signs of activity. Stages in the reduction division have been observed in all plants studied and show no unusual features. On the completion of the first division a dyad is produced. These cells soon divide and produce the macrospores, which in every case are arranged in a linear order (Text-Figs. 11, 13, 14 and 15). In *Euphorbia thymifolia,* however, in addition to the linear tetrad of macrospores, T-shaped tetrads have also been observed (Text-Fig. 12). In a few cases, the two upper macrospores are somewhat obliquely oriented. Maheshwari and Chowdry's investigations on the gametophytic development of *Phyllanthus niruri* shows that "it (megaspore mother cell) undergoes the usual reduction divisions and produces a tetrad of megaspores or a row of three cells of which the lower two are megaspores and the upper an undivided dyad cell". A critical examination of a number of preparations showing this stage failed to corroborate their account.

The lowermost or the chalazal megaspore alone functions while the three upper degenerate in every instance (Text-Figs. 11, 13 and 15). The course of degeneration appears to be from above downwards. In preparations showing later stages, the degenerated products could be seen as dark shapeless masses capping the functional megaspores.

(*e*) *The Development of the Female Gametophyte*

The functional megaspore increases in size and the cytoplasm becomes vacuolated, the vacuoles appearing mostly towards the periphery. It is interesting to note that in *Euphorbia thymifolia* two well-defined vacuoles occur above and below the nucleus which is placed centrally.

The binucleate stages are normal but a single instance of abnormality was observed in *Trewia nudiflora* where during the division of the two nuclei the sac was found to have elongated considerably measuring approximately 96·25 μ in length (Text-Fig. 16), its normal length at this stage being approximately 36·26 μ. By two successive divisions of the two nuclei an 8-nucleate embryo-sac is produced, four nuclei being oriented at each of the two ends of the embryo-sac. In *Putranjiva Roxburghii,* at the 8-nucleate stage, the nuclei at the chalazal end lie in a linear order, the uppermost one which functions as a polar fusion nucleus being alone separated by a wall (Text-Fig. 22). A similar separation of the nuclei destined to be the antipodal cells has also been observed, in *Phyllanthus niruri* (Text-Fig. 17) and *Euphorbia thymifolia* (Text-Fig. 19).

The mature embryo-sac shows the usual organization with the egg apparatus, the polar fusion nuclei and the three antipodals. These are differences, however, in certain details and as such it is proposed to describe each of them separately.

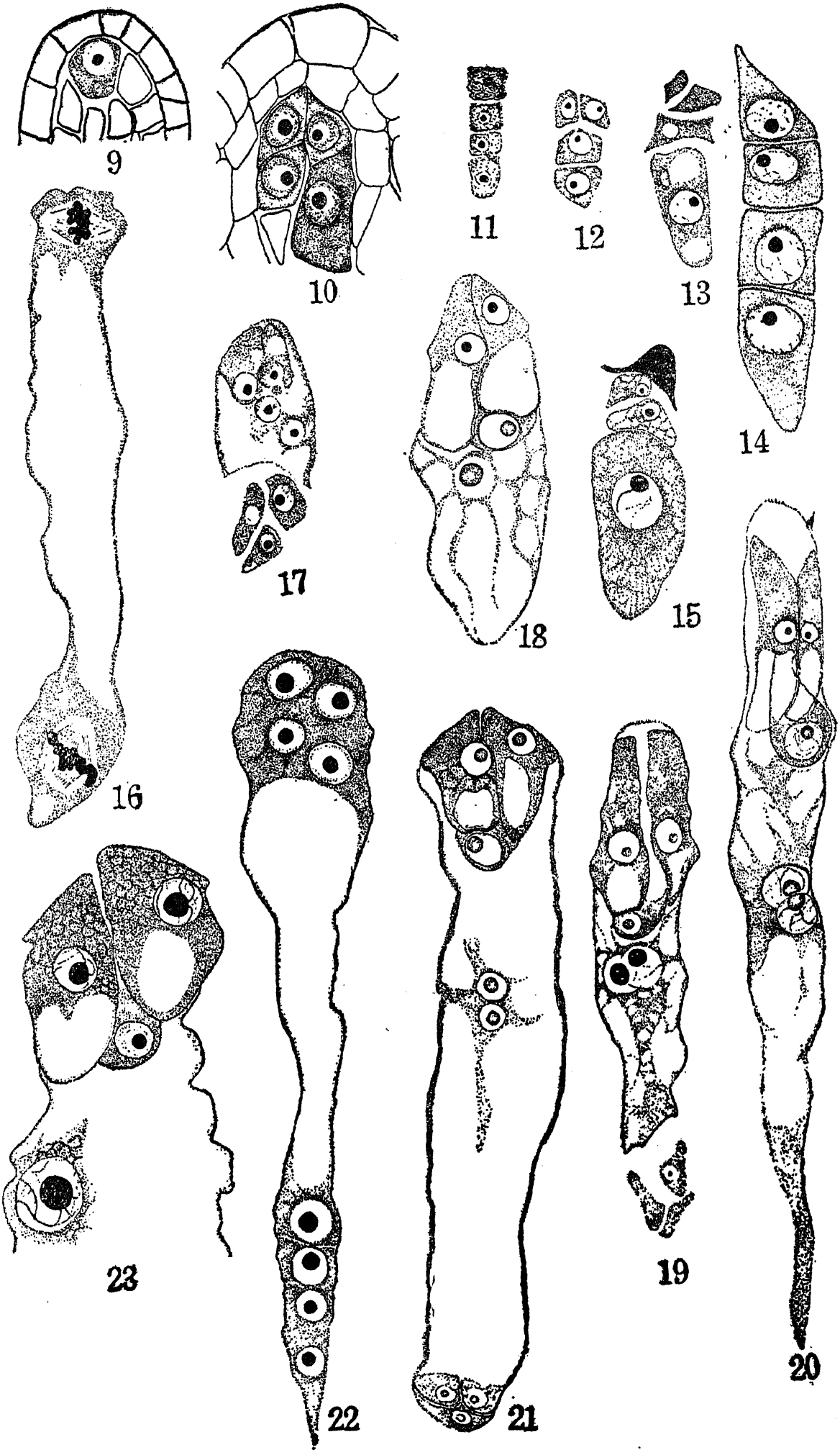
(*a*) *Putranjiva Roxburghii* Wall.—The mature embryo-sac is very much elongated approximating 192·5 μ in length. The synergids are distinctly hooked with the nuclei placed near the central region and above the large vacuoles which lie towards the base. The tip of the synergids shows the presence of innumerable small vacuoles giving the appearance of a honey-comb-like structure. The egg lies below and between the synergids with a vacuole at the top and nucleus embedded in a dense cytoplasm at the base. The secondry nucleus appears to be comparatively large lying somewhat close to the egg apparatus and embedded in a dense cytoplasmic mass. The antipodals have cytoplasmic walls and are generally arranged one above the other in a linear row. They do not show any signs of degeneration when the gametophyte is fully mature (Text-Figs. 23).

(*b*) *Trewia nudiflora* Linn.—The mature embryo-sac measures approximately 148·5 μ in length. As in *Putranjiva Roxburghii,* the synergids are hook-shaped and the egg apparatus shows no unusual features. The two polar fusion nuclei lie very close to each other near the central region of the embryo-sac, but have not been observed to fuse. The antipodal cells occupy the chalazal end of the embryo-sac (Text-Fig. 21).

(*c*) *Phyllanthus niruri* Linn.—The mature embryo-sac measures approximately 45·0 μ in length and appears to be 4-nucleate on account of the degeneration of the three antipodal cells which takes place earlier (Text-Fig. 18). The synergids are slightly hook-shaped and have the usual organization. Critical examination of a number of preparations shows that the egg always lies below one of the synergids and is not centrally placed. The secondary nucleus lies very close to the egg, its nucleus being comparatively large.

It should be pointed out in this connection that after the degeneration of the antipodal cells, the embryo-sac increases considerably in size and the chalazal end of the sac rounds off giving no indication whatsoever of the presence of the antipodals. Thus Arnoldi[1] who had failed to observe the earlier stages regarded it as a monosporic 4-nucleate type.

(*d*) *Euphorbia thymifolia* Burm.—The mature embryo-sac measures approximately 126·5 μ in length. The synergids in this case are somewhat elongated with blunt tips. The egg apparatus shows the usual arrangement of the nuclei and the vacuoles. In all the preparations observed the polar fusion nuclei appear in a state of fusion. The antipodals are cut off



Text-Figs. 9–24. Explanation in text. *Euphorbia thymifolia.*—Figs. 9, 12, 13, 19 and 20 *Putranjiva Roxburghii.* Figs. 10, 14, 22 and 23. *Trewia nudiflora.* Figs. 15, 16 and 21 *Phyllanthus niruri.* Figs. 11, 17 and 18. Fig. 21. × 950 ; the rest × 1540.

as separate cells (Text-Fig. 19). After their degeneration the embryo-sac elongates further (Text-fig. 20).

Summary

This paper gives a comparative account of the development of the ovules, macrospores and the female gametophyte of some members of the *Euphorbiaceæ, viz., Putranjiva Roxburghii, Trewia nudiflora, Phyllanthus niruri,* and *Euphorbia thymifolia.*

1. The ovules are anatropous and bitegmic.

2. In *Putranjiva Roxburghii* and *Trewia nudiflora* the integuments take part in the formation of the long micropyle whereas in *Phyllanthus niruri* and *Euphorbia thymifolia* no true micropyle is formed as the 'nucellar beak' lies in between the integuments.

3. Nucellar beaks have been noted in *Phyllanthus niruri* and *Euphorbia thymifolia.* In the former plant the beak remains extruded and curves towards the placental surface before the development of the female gametophyte, while in the latter it retains its original erect position from the beginning.

4. The presence of an obturator is reported in *Putranjiva Roxhurghii, Trewia nudiflora* and *Euphorbia thymifolia.* The obturator is massive. The outermost cells composing the obturator of *Euphorbia thymifolia* are hair-like and are uninucleate.

5. The single hypodermal archesporial cell gives rise by division to a megaspore mother cell and a cover cell in *Eurphobia thymifolia. A* multicellular archesporium has been noted in *Putranjiva Roxburghii* from which a single megaspore mother cell is formed. The megaspore mother cell is deep-seated in *Putranjiva Roxburghii, Trewia nudiflora and Phyllanthus niruri.*

6. A linear tetrad of macrospores has been observed in all the cases. In *Euphorbia thymifolia* T-shaped tetrads have also been observed.

7. In all the plants the chalazal megaspore alone functions, the others degenerating from above downwards.

8. The development of the embryo-sac is of the normal type. In *Phyllanthus niruri* the mature embryo-sac is 4-nucleate while in *Euphorbia thymifolia* it is 5-nucleate.

9. Hooked synergids have been observed in *Putranjiva Roxburghii, Trewia nudiflora* and *Phyllanthus niruri.* In *Euphorbia thymifolia,* it is somewhat elongated with blunt ends. Secondary nucleus was observed

in *Putranjiva Roxburghii* and *Phyllanthus niruri*. In *Trewia nudiflora* and *Euphorbia thymifolia* the polar nuclei remain close to each other but have not been observed to fuse. Antipodals are ephemeral in *Phyllanthus niruri* and *Euphorbia thymifolia*. In *Putranjiva Roxburghii* and *Trewia nudiflora* they persist.

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