# STUDIES IN THE MALPIGHIACEÆ.

## 2. Structure and development of the ovules and embryo-sacs of *Malpighia coccifera* Linn. and *Tristellateia australis* Linn.*

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A list of species of the Malpighiaceæ, which have been embryologically studied up to this time, is given below :—

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| Tribe I Malpighieæ | *Malpighia urens* L., Schürhoff (1924) and Stenar (1937). |
| | *Malpighia punicifolia* L., Narasimhachar (1938). |
| | *Malpighia glauca* Poir., Subba Rao (1939). |
| | *Malpighia coccifera* L., Schürhoff (1924). |
| | *Bunchosia nitida* Jacq., Schürhoff (1924). |
| | *Galphimia gracilis* L., Stenar (1937). |
| Tribe II Banisterieæ | *Heteropterys* sp., *Banisteria* sp., *Stigmatophyllum* sp. } Braun (1859-60). |
| | *Stigmatophyllum aristatum* L., Subba Rao (1940). |
| | *Banisteria laurifolia* L., Subba Rao (1940). |
| Tribe III Hireæ | *Hiptage Madablota* Gaertn., Subba Rao (1940). |
| Tribe IV Gaudichaudieæ | *Aspicarpa longipes*, *Aspicarpa lanata*, *Aspicarpa hirta* } Ritzerow (1908). |

Of these, *Malpighia glauca* Poir. (Subba Rao, 1940) and *Galphimia gracilis* L. (Stenar, 1937) show a bisporic eight-nucleate embryo-sac (*Allium*-type); the rest have a tetrasporic sixteen-nucleate embryo-sac of the *Penæa*-type. *Heteropterys* sp., *Stigmatophyllum* sp., and *Banisteria* sp. (Braun, 1860), *Hiptage Madablota* Gaertn. (Subba Rao, 1940), *Aspicarpa longipes, A. lanata* and

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*A. hirta* (Ritzerow, 1908) show nucellar polyembryony. In *Malpighia punicifolia* L. (Narasimhachar, 1938), however, a single embryo near the micropylar end has been reported.

The structure and development of the ovules and embryo-sacs of *Malpighia coccifera* L. and *Tristellateia australis* L. are described in the present paper.

## Material and Methods.

The present investigation was taken up in the year 1937. The material was collected on bright sunny days from the Government Lal Bagh Horticultural Gardens, Bangalore City, and fixed in Bouin's fixing fluid. After immersion for 24 hours in the fixative it was transferred directly to 50% alcohol. The usual methods of dehydration and embedding in paraffin were followed. Sections were cut 10–14 microns thick and stained with iron-alum hæmatoxylin.

### *Malpighia coccifera* Linn.

This small erect undershrub, about 2-3 feet high, is grown for its attractive flowers. The stamens are all perfect and the gynæceum, which is not as pubescent as in *Hiptage Madablota*, is composed of three carpels each having its own style and stigma. The ovary, unlike that of *Stigmatophyllum*, *Banisteria*, *Hiptage* and others, develops into a fleshy drupe.

*Ovule:* The pendulous ovule originates from the axile placenta as in the other members. It ultimately assumes an anatropous form. The integuments are initiated at approximately the same time, but the inner remains very short and the micropyle is formed by the outer integument only (Fig. 3). The development of the ovule is similar to that of *Malpighia urens* (Stenar, 1937). The inner integument is about 3-4 layers and the outer is 2-3 layers thick. The terminal part of the outer integument, towards the placenta, is thrown into folds which may be due to want of space in the locule for the proper stretching of this region. The identity of the integuments is almost lost during later stages. The outer layer of cells of the outer integument is heavily laden with tannin (Fig. 2). The funicle is massive and long. The orientation of the ovule can be clearly understood from fig. 3, in which the dotted line represents the funicle.

*Development of the embryo-sac:* Schürhoff (1924), who investigated this plant previously, states that there is no multiple archesporium. But as in *Malpighia punicifolia* (Narasimhachar, 1938), *Hiptage Madablota*, *Banisteria laurifolia* and *Stigmatophyllum aristatum* (Subba Rao, 1940) it is observed that the archesporium which differentiates very early in the young nucellus usually consists of 3-4 cells (Fig. 1). The hypodermal archesporial cell cuts off a parietal cell which in turn divides 2-3 times. Often 3-4 parietal cells are observed just above each archesporial cell. The epidermis and the parietal cells divide in all planes thus giving rise to a big nucellar-beak which forms a noteworthy feature in all members of the family hitherto studied (Figs. 2 and 3). The cells towards the interior of this nucellar-beak in this plant are comparatively larger but poorer in cytoplasmic contents (Fig. 3). It cannot be definitely stated whether the parietal cells or the epidermal cells contribute more towards the formation of this big and prominent nucellar-beak.

The nucleus of the megaspore mother cell is surrounded by a rich, densely staining and evenly distributed cytoplasm. After undergoing the usual reduction divisions (Figs. 1 and 4) it gives rise to four free and crosswise-arranged nuclei; they are all connected to each other by spindle fibres as in the other members of the family investigated by me, except *Malpighia glauca* which has a bisporic embryo-sac.

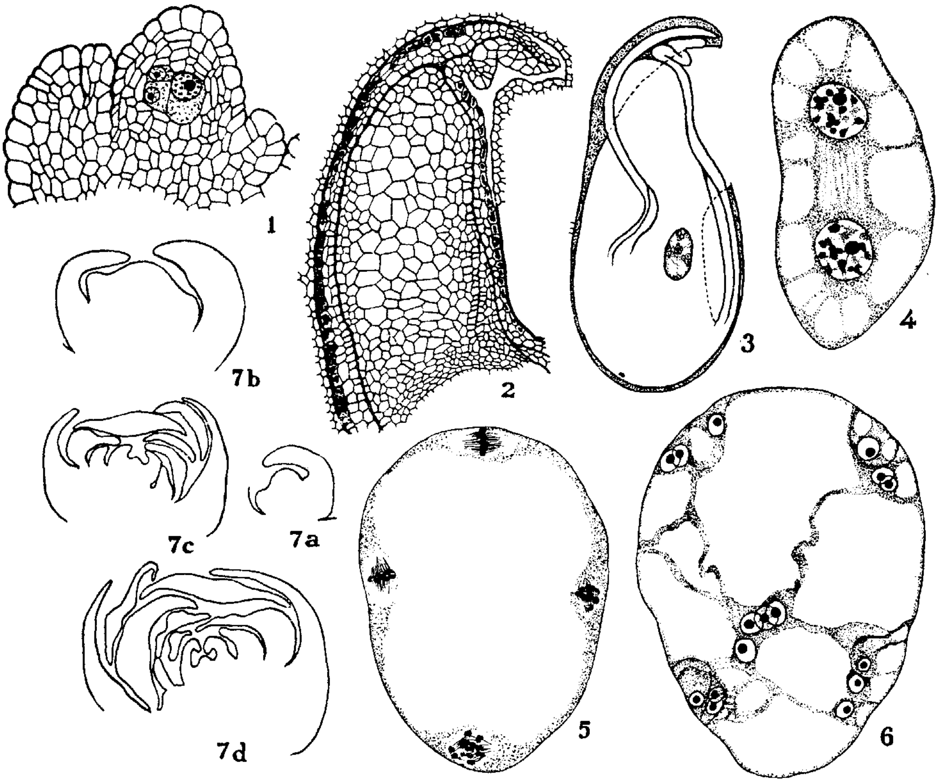
As the embryo-sac enlarges, vacuoles appear and the four megaspore nuclei are now pushed to the periphery where they undergo two simultaneous divisions (Fig. 5) to form a sixteen-nucleate embryo-sac composed of four quartets. These observations confirm what Schürhoff (1924) has already recorded for the plant.

The organisation of the mature embryo-sac is very similar to the *Penæa*-type, but the three cells organised at the four poles are not definitely distinguishable into egg and synergids. The secondary nucleus, as is usual, is formed by the fusion of four nuclei in the centre, one coming from each quartet. The antipodal group of cells is the first to degenerate and is later followed by the other three groups of cells (Fig. 6). Finally, the embryo-sac collapses, as in *Combretum Pincianum* (Mauritzon, 1939) and the whole ovule shrivels up. Sometimes the ovary is seen to develop further giving rise to a seedless drupe. Since the plant has not been producing seeds in Bangalore the embryonal development could not be followed.

## *Tristellateia australis* Linn.

The vegetative and floral parts of *Tristellateia australis* L. are broadly similar to those of *Hiptage Madablota*, but the former is easily distinguished by having a style corresponding to each of the three carpels. Of the three styles it is often observed that only one is well-developed and the other two are rudimentary. Sometimes, all the three styles are of different size, one of them being reduced to the size of a minute projection on the carpel. The stigma is very small and knob-like. The plant usually flowers during August and September but the duration of flowering is very short. The floral parts develop in the usual acropetal succession, viz., calyx, corolla, stamens and carpels (Figs. 7*a*–*d*).

*Development of the ovary and ovule:* The tricarpellary ovary arises as a hemispherical mass of meristematic cells from the tip of the floral axis after the latter has formed all the other parts. The primordia of the three carpels develop simultaneously as a ring of meristematic cells which grows and bends inwards at the distal end and fuses with the projection of the




Figs. 1–6. *Malpighia coccifera* L.
Fig. 7. *Tristellateia australis* L.

Fig. 1. L.S. ovule showing integuments, multiple archesporium and a number of parietal cells. ×160.

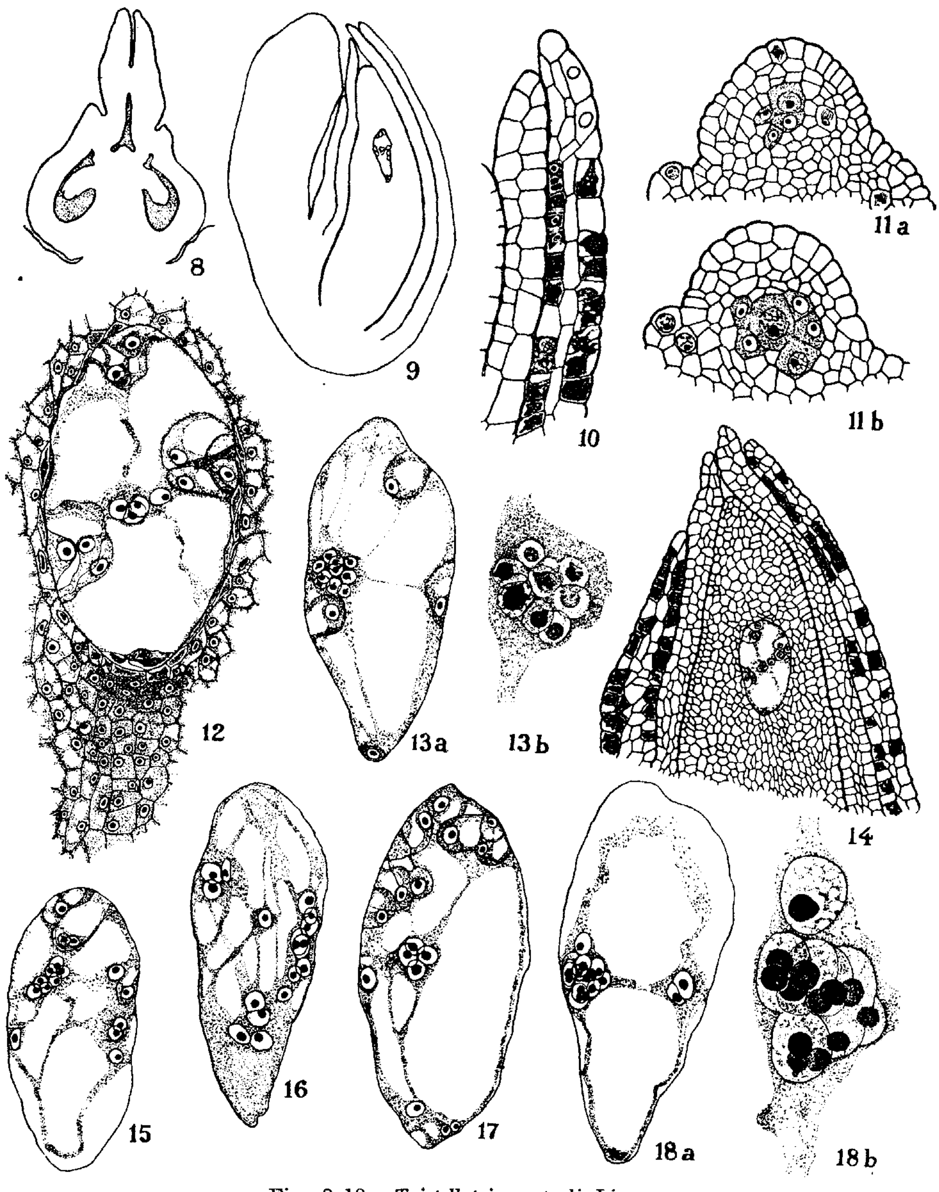
Fig. 2. L.S. of nucellar-beak. Note the larger cells in the centre and presence of tannin in the outer layer of the outer integument. ×65.

Figs. 4–6. Two-, four-nucleate (the nuclei in this are in mitosis) and mature embryo-sacs. Figs. 4 & 5 ×365. Fig. 6 ×182·5.

Figs. 7*a*, *b*, *c*, & *d*. Early stages in the development of the flower. Figs. 7*a* & *b* ×32·5, *c* & *d* ×16·7.

conical axis (Figs. 7*c*, *d* and 8). The incurved carpellary walls continue to grow from their outer surface at the bent region and again fuse a little further up enclosing a small space which is finally closed at both ends (Fig. 8). Three regions, one corresponding to each carpel, grow and get differentiated into the three styles and stigmas. Later on the three locules are formed by the appearance of three septa.

As the locules are formed, the three pendulous ovules begin to develop from the axile placentæ. In later stages they become anatropous (Figs. 8 and 9). In development and orientation they are similar to *Malpighia urens* (Stenar, 1937).




Figs. 8–18. *Tristellateia australis* Linn.

Fig. 8. L.S. carpel showing the development of the ovules and styles. ×16·7.

Fig. 9. L.S. ovule (diagrammatic). ×32·5.

Fig. 10. Portion of integuments enlarged to show tannin contents. The inner integument and the middle layer of the outer are free from tannin. ×182·5.

Fig. 11*a*. L.S. nucellus showing multiple archesporium, one epidermal cell is in division. ×160.

Fig. 11*b*. Same. One archesporial cell has enlarged. ×160.

Fig. 12. Mature embryo-sac. Note the mass of nutritive cells at the chalazal end. $\times 182 \cdot 5$.

Fig. 13*a*. Embryo-sac with four peripheral cells and a group of polar nuclei. $\times 182 \cdot 5$.

Fig. 13*b*. Enlarged view of the polar nuclei (probably ten) of fig. 13*a*. $\times 365$.

Fig. 14. Nucellar-beak with enveloping integument and embryo-sac lying within. $\times 65$.

Fig. 15. Mature embryo-sac showing absence of antipodal cells and a lack of definiteness in the arrangement of the nuclei. 5 nuclei are seen to the right and one to the left leaving 7 to fuse in the centre. $\times 182 \cdot 5$.

Fig. 16. Of the 16 nuclei in the embryo-sac, 8 are placed towards the right, 4 towards the left and 4 at the chalazal end. One nucleus from the left has moved towards the centre. $\times 182 \cdot 5$.

Fig. 17. 16-nucleate stage with 8 cells organised near the micropylar end. $\times 182 \cdot 5$.

Fig. 18*a*. Abnormal embryo-sac with 13 nuclei to the left and 2 to the right; the 16th nucleus could not be traced. $\times 182 \cdot 5$.

Fig. 18*b*. A portion of fig. 18*a* enlarged to show the 13 nuclei on the left side. $\times 547 \cdot 5$.

The integuments make their appearance quite early, the inner being the first to differentiate. Both of them are about 2-3 cells thick for most of the distance (the outer consists of more than three layers towards its base) and can be made out distinctly even during the embryo-sac stage in the ovule. The inner integument which projects beyond the outer and reminds one of a similar feature in *Hiptage Madablota* (Subba Rao, 1940) forms the micropylar boundary. Tannin appears in the cells of the inner and outer layers of the outer integument as in *Malpighia coccifera* and the middle layer is devoid of the same (Fig. 10).

Just as in the other members of the family studied by me, the nucellar cells at the micropylar end divide in quick succession and develop into a short beak which protrudes into the micropyle (Figs. 9 and 14). The chief difference, however, is that the beak is here surrounded by the inner integument. Unlike *Malpighia coccifera* all the cells composing it are here of an approximately similar size (Fig. 14).

*Megasporogenesis:* The multiple archesporium (Figs. 11*a* and *b*) consisting of 3-4 cells differentiates very early, even before the appearance of the integuments. Of these archesporial cells only one was observed to develop further, although in *Banisteria laurifolia* and *Hiptage Madablota* (Subba Rao, 1940) more than one may occasionally continue to grow. In *Banisteria* two sixteen-nucleate embryo-sacs were once seen in the same ovule.

*Development and structure of the embryo-sac:* The embryo-sac seems to arise in the normal way described for the *Penæa*-type (Stephens, 1906). Eight- and sixteen-nucleate stages of the embryo-sac have been observed. Earlier stages could not be found although plenty of material was collected for investigation. This may be due, in some measure, to the rapidity with which the whole development takes place. The mature embryo-sac conforms to the type described for *Hiptage, Banisteria* and *Stigmatophyllum* (Subba Rao, 1940), *Malpighia punicifolia* (Narasimhachar, 1938) and *M. urens* (Stenar, 1937). There are four groups of three cells each and four nuclei coalescing in the centre to form the secondàry nucleus. At the chalazal region there are some densely staining cells which probably have a nutritional function (Fig. 12). The organisation of the three micropylar cells to form an egg-apparatus seems to be more marked in this plant than in the other forms previously investigated by me (Figs. 12, 15 and 17). The lateral groups may also show a differentiation into egg and synergids but the three nuclei or cells at the chalazal end degenerate very early (Fig. 12).

Failure of fertilisation has already been reported in *Hiptage Madablota* (Subba Rao, 1940) and the causes for such a failure have also been enumerated. Certain stages in the pollen formation have been observed in *Tristellateia australis* and the development has been found to be normal. Considering the normal development of the gametophytes and the normal organisation of the three micropylar cells, it seems probable that pollination and fertilisation may take place in the usual way in this plant provided the environmental and physiological processes are normal.

Some variations in the development and organisation of the mature embryo-sac that were encountered in this plant are described below.

Fig. 13*a* is interesting in having only four peripheral cells. Ten polar nuclei were distinctly seen making a total of 14 nuclei in the embryo-sac. Since the number of nuclei in the group is large and happens to overlap one another (Fig. 13*b*) it is probable that two have been missed making a total of 12 polars.

Fig. 15 shows a mature embryo-sac with three micropylar cells, five nuclei to the right, seven in the centre and one nucleus to the left. The chalazal region is completely devoid of nuclei.

Fig. 16 may be considered to be an earlier stage in the formation of the type of embryo-sac represented in fig 15. It presents eight nuclei to the right, four to the left and four at the chalazal end. The micropylar region is free from cells, or even nuclei. If three, of which one has already moved, nuclei from the left, three from the right and one from the chalazal end migrate to the centre the embryo-sac will be similar to that in fig. 15, but with a reversed polarity.

Fig. 17 is a sixteen-nucleate stage with eight cells near the micropylar end, one free nucleus to the left and three antipodal nuclei. This condition has apparently resulted from a close approximation of the lateral triads to the micropylar end; only one nucleus on the left has failed to move up or become cut off by a wall.

Fig. 18*a* is difficult to interpret. The embryo-sac has thirteen nuclei (Fig. 18*b*) to one side and two to the other which are partially merged into each other. It seems that due to some physiological disturbance further organisation of the embryo-sac failed to take place. A detailed discussion of this embryo-sac seems futile and the observation is recorded for future reference.

Cases showing either reduced or increased number of nuclei in the embryo-sacs were not met with during the present investigation. As a rule all the

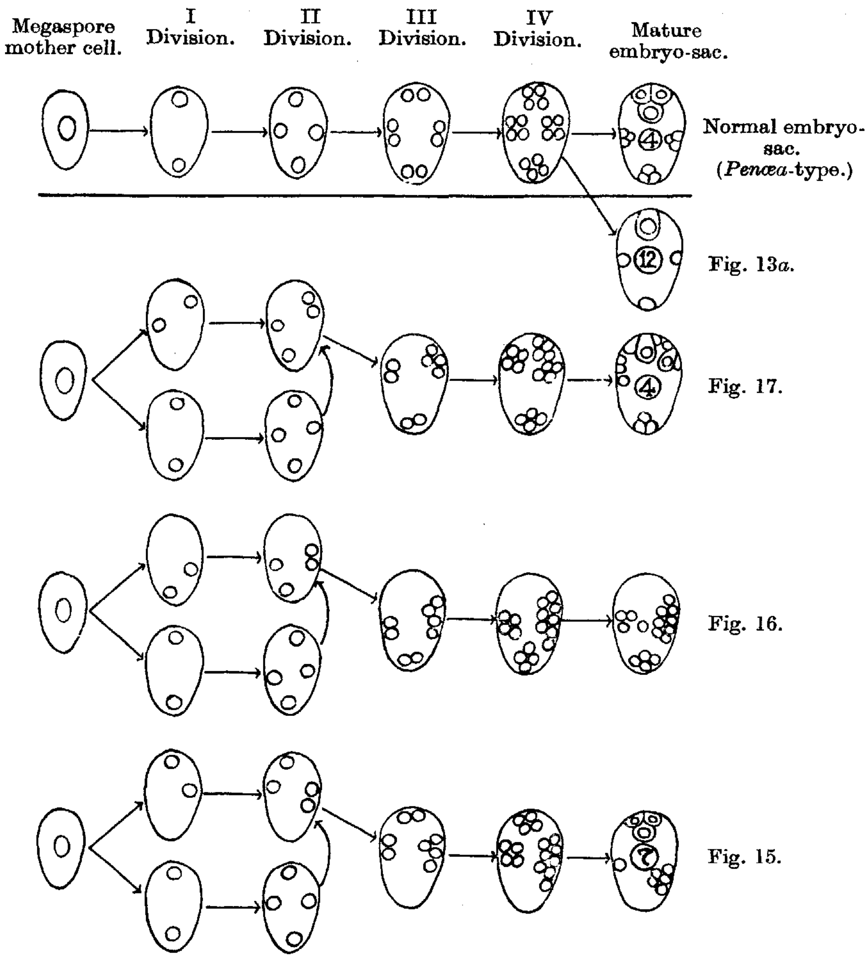



FIG. 19. Diagram to illustrate the development of the normal and probable derivation of abnormal embryo-sacs described in the text.

embryo-sacs presented sixteen nuclei but the organisation of the mature embryo-sacs presented some variations.

The chart presented above shows the normal development and also indicates the possibilities of derivation of the abnormal embryo-sacs described above (Fig. 19).

## CONCLUSIONS.

The family Malpighiaceæ presents two different types of embryo-sac development, the *Penæa*- and *Allium*-types. Among the variations that have been observed in the organisation of the mature embryo-sac the one pictured in fig. 13*a* is of special interest. It shows certain resemblances to the *Plumbago*-

type (Haupt, 1934 and Dahlgren, 1937) on the one hand and *Acalypha indica*-type (Maheshwari and Johri, 1940) on the other. The main points of resemblance with *Acalypha indica* are its tetrasporic origin, sixteen nucleate nature and tetrapolar organisation. The only difference lies in the organisation of the peripheral groups. *Acalypha indica* has four two-celled peripheral groups and eight free nuclei in the centre, whereas here the peripheral groups are represented by a single cell each leaving twelve free nuclei for the centre. The similarities with the embryo-sac of *Plumbago* are the tetrasporic origin and tetrapolar organisation of both the sacs and the presence of four peripheral cells in each. The differences lie in the total nuclear content of the two embryo-sacs and the number of nuclei that function as polars, both of which may be attributed to the fact that in *Plumbago* there is one division less between the megaspore mother cell and the egg as compared to the condition in the Malpighiaceæ. Thus, it is highly probable that this embryo-sac may occupy an intermediate position between *Plumbago* (Haupt, 1934; Dahlgren, 1937) and *Acalypha indica* (Maheshwari, 1940). If the embryo-sac exhibiting this type of organisation is met with more frequently, a regular transitional series starting with *Plumbago*—with one more division intervening—can be arranged as illustrated below (Fig. 20).

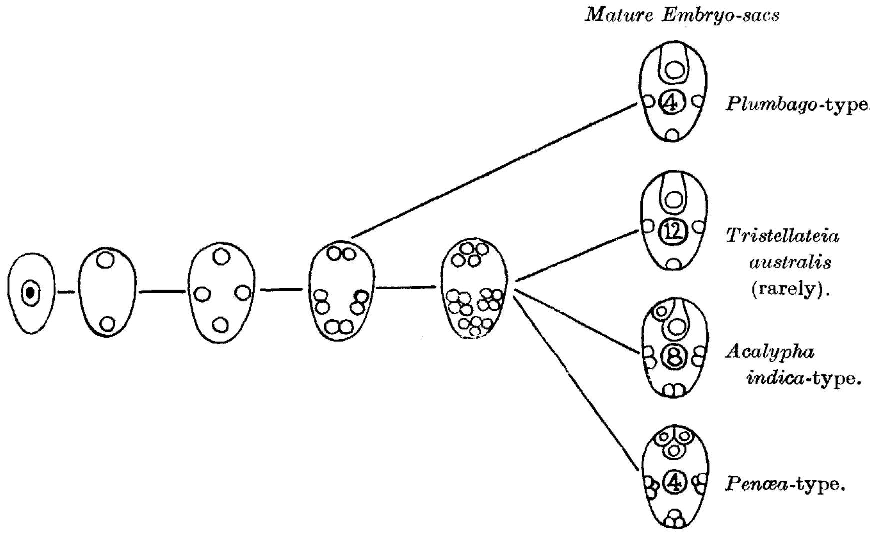



FIG. 20. Relationships of the embryo-sacs of *Plumbago, Tristellateia australis, Acalypha indica* and *Penœa*.

Coming to the bigger problem of the origin of the various types of abnormal embryo-sacs the author concurs with the view expressed by Dr. Maheshwari (1939) that 'the monosporic eight-nucleate embryo-sac is the fundamental type in angiosperms and that all the others are but modifications of this type (see also Schnarf, 1936), derived by a decrease in the number of divisions taking

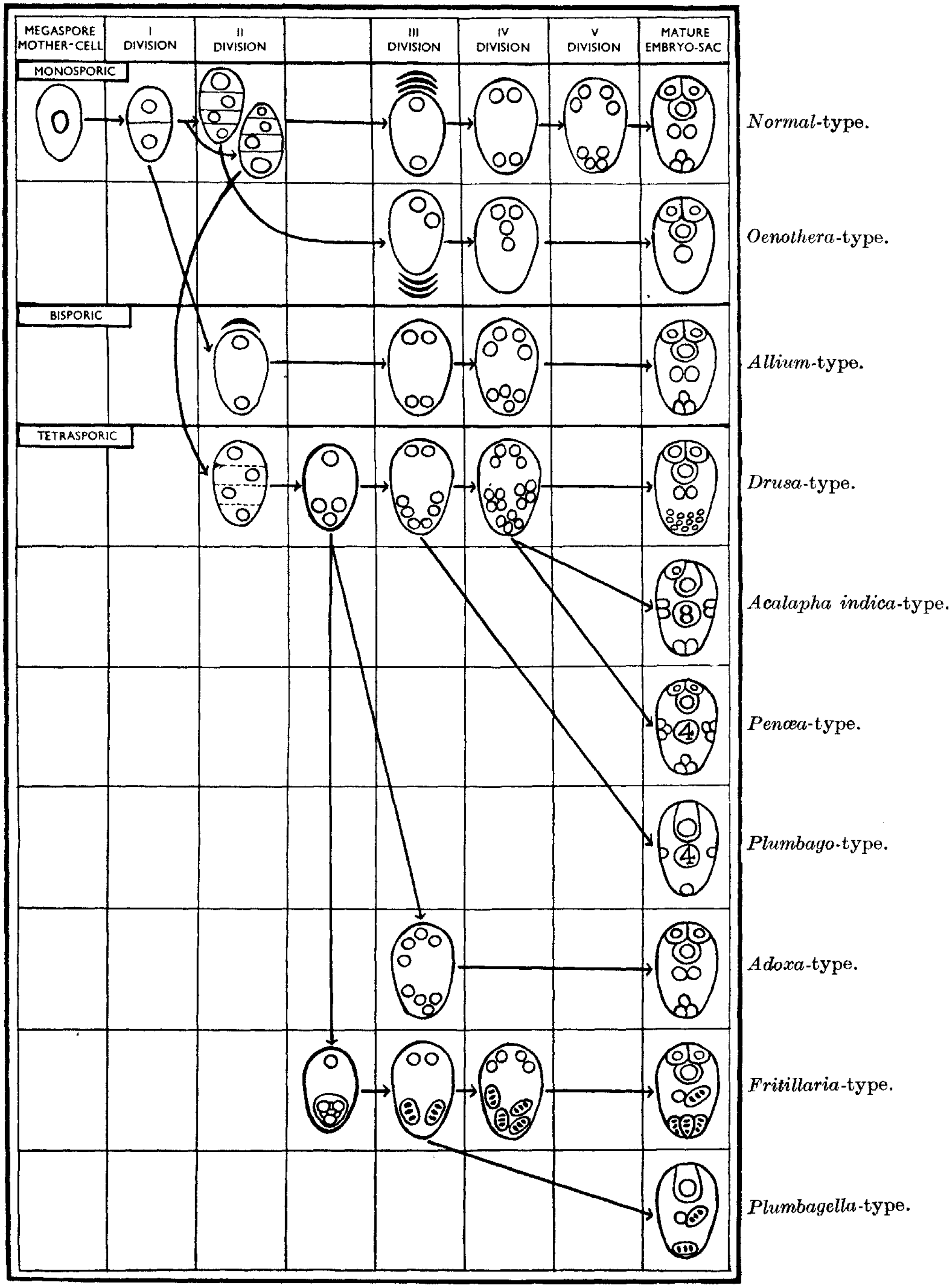



FIG. 21. Homology of angiosperm embryo-sacs. (Shows the number of divisions intervening between the megaspore mother cell and the mature embryo-sac.)

place between the megaspore mother cell and the egg or by an increase in the number of megaspores taking part in the formation of the sac'.

Fig. 21 shows the methods by which the different types of embryo-sacs may have been derived from the normal monosporic eight-nucleate type.

## Summary.

Two horticultural plants, *Malpighia coccifera* L. and *Tristellateia australis* L. have been investigated.

The development of the ovule resembles that of *Malpighia urens*, previously described by Stenar (1937).

A multiple archesporium was observed in both the plants but only one cell usually develops further. The developing mother cell becomes deeply placed on account of the formation of a massive parietal tissue constituting the nucellar-beak.

The micropyle is formed by the outer integument in *Malpighia coccifera*, and by the inner in *Tristellateia australis*.

The embryo-sacs of both the plants develop after the *Penæa*-type as described for the other plants of the family except *Malpighia glauca*. The mature gametophytes in both have tetrapolar groups of three cells each and four nuclei uniting in the centre to form the secondary nucleus.

A number of variations in *Tristellateia* and their probable origin have been discussed in the text.

The homology of the different types of the embryo-sacs in angiosperms is briefly discussed and illustrated.

In conclusion I wish to express my indebtedness to Dr. M. A. Sampathkumaran, M.A., Ph.D., S.M. (Chicago), Head of the Department of Botany, Central College, Bangalore, for kind guidance and encouragement during the present investigation, and to Mr. C. V. Krishna Iyengar, M.Sc., for valuable suggestions throughout the course of study. My sincere thanks are also due to Dr. P. Maheshwari, D.Sc., F.A.Sc., F.N.I., Head of the Biology Department, Dacca University, whose suggestions and criticisms were of immense help in the preparation of this paper.

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* Quoted from Schnarf (1929) and Schürhoff (1926).