DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND ENDOSPERM IN HABERLEA RHODOPENSIS FRIV.

(Contribution to the knowledge of the life-history of Gesneriaceae.)

— Dr. **Ljubiša M. Glišić.** —
(Beograd)

The plants furnishing the material for the present study are native of Bulgaria. When one considers the great importance of *Haberlea* as a relic of the tertiary flora of the Balcan Peninsula, it is much surprising the dearth of information relating to its morphology as well as its ecology. The main aim of the present paper would be to work out the development of the ovule, embryo-sac and endosperm of this plant, and to make so a little advance in our knowledge on *Haberlea* as already made by Košanin (11, 12), Glišić (8), Fritsch (7), Hayek (9) and others on *Ramondia.*

As known, the family of Gesneriaceae is represented on the Balcan Peninsula by three endemic genera: *Ramondia, Haberlea* and *Jankaea.* They are all relics which survived the tertiary period, and are presently strongly adapted to the special life conditions. They are to be considered as our own Balcan plants, and it was our duty to undertake a study in order to understand and explain their life.

Pollinations were made by hand (1925) in order to secure the fertilization. In some intervals afterwards the ovaries were collected at various stages of development and immediately fixed in a mixture of alcohol absolutus and acidum aceticum glaciale. Paraffin (m. p. 52—54°) sections were cut 10—15 μ. thick. For most of the staining Heidenhain's iron-alum haematoxylin was used, but haematein-eosin and Flemming's safranin-gentiana violet-orange combinations were also found to be very useful and satisfactory.

The ovules are similar in structure with those given for *Ramondia* (8) and *Klugia* (17). The ovaries consist of two carpels, bearing two rows of ovules each. A single integument is formed. The nucellus is of the tenuinucellate type (syndermal, according to Dahlgren's (6) new terminology) consisting of a single layer of cells. No tapetal cell is cut off. The development of the embryo-sac is in the normal manner.

The archesporial cell is originating hypodermally. Owing to the lack of very young stages in preparations, I was not able to decide whether one or more archesporial cells take their origin in the hypodermal layer. But in every case observed the division of the archesporial cell resulted in the formation of four megaspores in an axial row separated by definite cell walls. The innermost megaspore of this linear tetrad functions as the fertile megaspore: it develops into the embryo-sac. The other three are gradually disintegrating (Text Fig. 1.). There is no exception to this rule. The disintegrating megaspores remain recognizable for a long time afterwards as a darkly staining group over the young embryo-sac, later being pressed between the integumental tapetum and endosperm tissue (Text Fig. 2., 3.).

The product of the first gametophyte division results in a binucleate embryo-sac (Text Fig. 2.). A large central vacuole appears between two nuclei. At this stage the embryo-sac grows considerably at its micropylar end, and passes by the other three sisters. Continued growth of the embryo-sac results in the upward pushing of the nucellar epidermis whose cells become compressed and show much indication of disintegration. The ensuing division shows two nuclei at each end of the sac. Preceding the third division, a considerable growth and lengthening of the embryo-sac occurs, the nucellar epidermis becomes finally broken at its micropylar end, the embryo-sac is

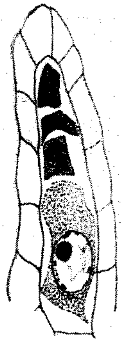


Fig. 1.
700 ×

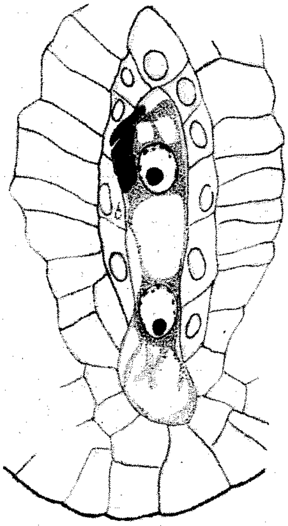


Fig. 2.
700 ×

entering the micropylar canal and extending into it. The third division takes place, and the eight nucleate stage is reached. Immediately afterwards the two polar nuclei are wandering toward each other, the egg-apparatus and three antipodals are organised at the opposite ends of the embryo-sac which is now complete. By this time the sac has slightly broadened at its micropylar end. A layer of well defined cells of the integumental tapetum is bounding the sac, only the region of the antipodals and the broadened micropylar and are free of it. The cells of the integumental tapetum differ from other neighbouring cells of the ovule, being richer in protoplasmic contents (Text Fig. 8.; Pl. II, Fig. 4.).

Several times two embryo-sacs have been observed in the same ovule. The origin of this abnormality could not be ascertained; whether two archesporial cells or two megaspores belonging to a single tetrad were in question, remains unanswered.

The polar nuclei are a long time lying against each other, and fuse at a moment immediately preceding or succeeding the process of fecundation of the egg. They remain for a long time at a conjugate stage.

The pollen tubes could be traced on their way to the micropyles. Through the greater part of their course they are travelling over the surface of the epidermis lining the stylar canal and placenta; they never enter the tissue. The entrance of the pollen tube through the micropyle (porogamy) and into the embryo-sac is clearly visible, the remnant of the tube usually persisting for a considerable time after the fecundation (Text Fig. 3.). The pollen tube containing the vegetative and both male nuclei enter the cavity of the ovary in form of a bundle. They are easily finding their way to the micropyles on account of the anatropous structure of the ovules whose micropyles are nearly in contact with the epidermis of the placenta. Normally one of the synergids disintegrates, this alteration being caused by the proximity of the pollen tube (Text Fig. 3.). Occasionally both synergids are represented only by a shapeless mass. At about this time three antipodals begin to disintegrate.

A very large number of the embryo-sacs were examined in order to discover the male gametes. They were somewhere hidden among the numerous highly granular and darkly staining bodies of the distintegrated synergid. The male nuclei could be always distinguished with certainty only in cases when lying against the egg nucleus or the secondary nucleus of the embryo-sac (Text Fig. 3.).

The actual union of the male and the ovum nuclei was observed very often. For a long time two conspicuous nucleoli were to be seen, the smaller one belonging to the male. While in the pollen tube, the male nuclei do not show any nucleolus, and it is during this process of union of the sexual nuclei that the male nucleolus is formed.

The second male nucleus had also many times been observed to lie against the secondary nucleus of the embryo-sac. And their actual union was ascertained; their fusion in the endosperm nucleus is complete.

By this time the embryo-sac has enlarged somewhat, since its contents seem not to be increased at all; it looks rather empty.

The process of fecundation may be held as quite normal.

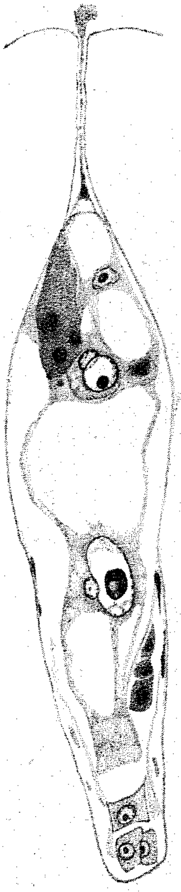


Fig. 3.
820 ×

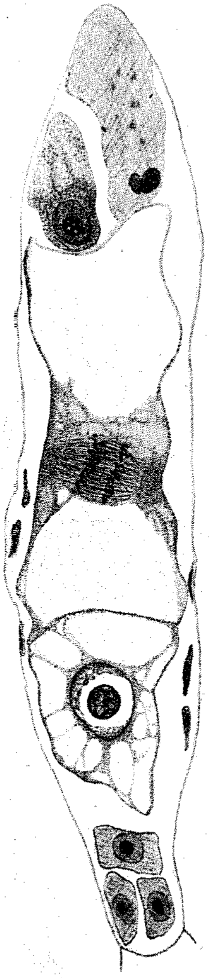


Fig. 4.
983 ×

Immediately following the fecundation, without any delay, the endosperm nucleus is preparing for division. The mitosis (Pl. I, Fig. 1.) is accompanied by the wall formation; therefore the endosperm is cellular *ab initio*. The first division is *transverse*, resulting in the formation of two unequal chambers, two primary endosperm cells (Pl. I, Fig. 2.). The smaller chalazal cell occupies one-third of the previous cavity of the embryo-sac. The transverse wall forms at the height of the destroyed nucellar epidermis, whose disintegrated cells are still recognizable by the remnant of their walls. From this point onwards the two chambers behave differently. The development of the endosperm tissue is from the chamber next to the micropyle; it undergoes repeated longitudinal and transverse divisions. The mitosis in the upper chamber is accompanied by a *longitudinal* cell wall (Text Fig. 4., 5.; Pl. I, Fig. 3.). The ensuing division of these two cells is *transverse*, thus resulting in the formation of four cells arranged in two rows in a single plane (Text Fig. 5.; Pl. II, Fig. 4.). The two uppermost of them seem to function as poorly developed micropylar haustories; they remain as such or may be divided once more transversally. The two lower cells lying above the chalazal cell represent the initials of the very endosperm tissue; they undergo repeated transverse and longitudinal divisions.

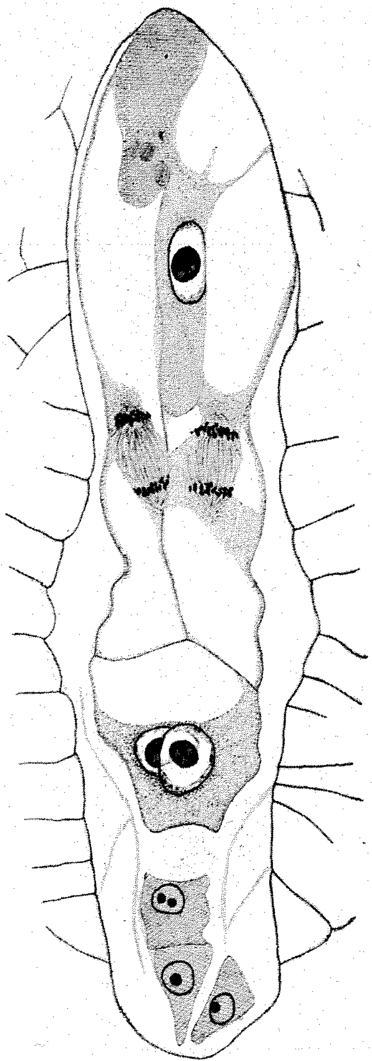


Fig. 5.
983 ×

The nucleus in the chalazal chamber divides too, but in the most of cases observed no cell wall is formed between them. This cell becomes *binucleate* (Fig. 6: a, b, c, d; Fig. 7 a). There is no trace of the cell plate. The binucleate condition remains till the end of the endosperm development. Both nuclei are lying side by side and show a strong tendency to unite (Fig. 6 d; Fig. 7 a). The lower part of the chalazal cell is filled with a denser cytoplasm staining more deeply with Heidenhain's iron-alum haematoxylin; in its upper end is regularly a large vacuole to be found. This cell shows much indication to function as the chalazal haustory, poorly developed as compared with such structures in other genera and species of Tubiflorae: Labiatae (16), Scrophulariaceae (14), Plantaginaceae (15). The process of union of nuclei

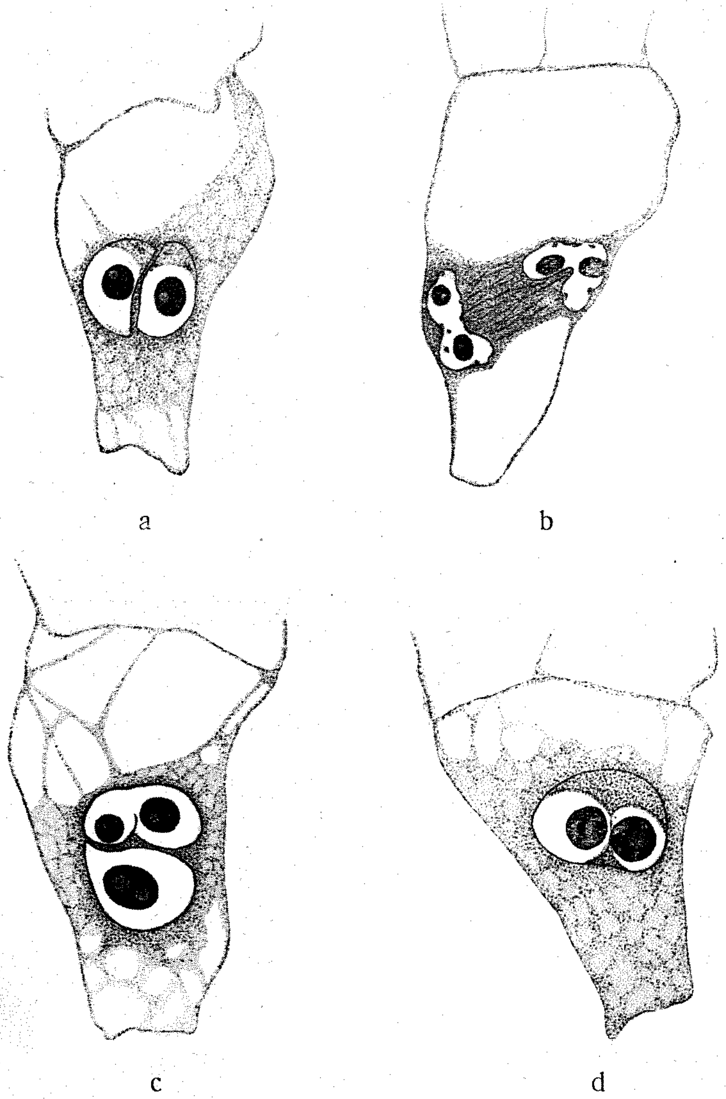


Fig. 6.
1475 ×

is never complete, the two nucleoli are always recognizable. The outline of nuclei becomes rather irregular and their constitution somewhat altered, caused by their function of nutrition. It happens often that their irregular shape is already indicated at the late telophasis (Fig. 6 b); they assume a biscuitlike form and contain two nucleoli each.

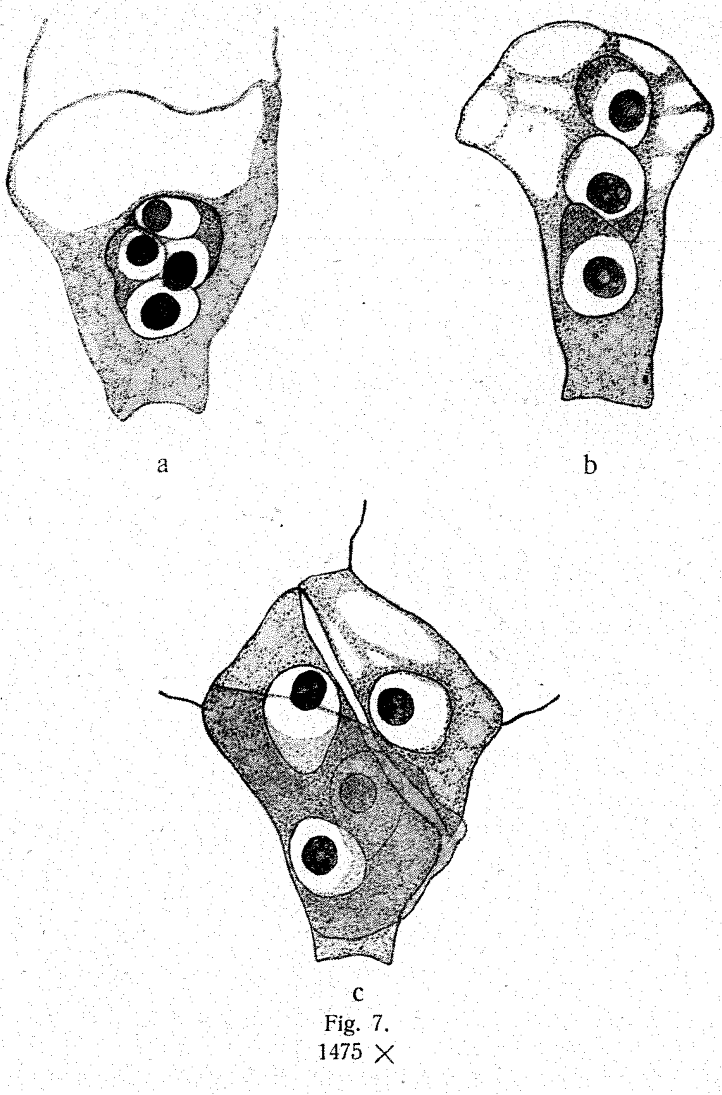

a b c

Fig. 7.
1475 ×

The above description of the development process of the chalazal cell may be considered as normal, occurring in great majority of cases. But many times could be ascertained that this is not the only mode; the development can take another line. Instead of remaining one-celled with two nuclei, the chalazal cell may often undergo a division, thus resulting in the formation of two cells at the chalazal end (Text Fig. 8.). The cell wall which separates the two cells is never longitudinal; it is usually more or less inclined and *oblique*, and very rarely horizontal. The cells contain one nucleus each.

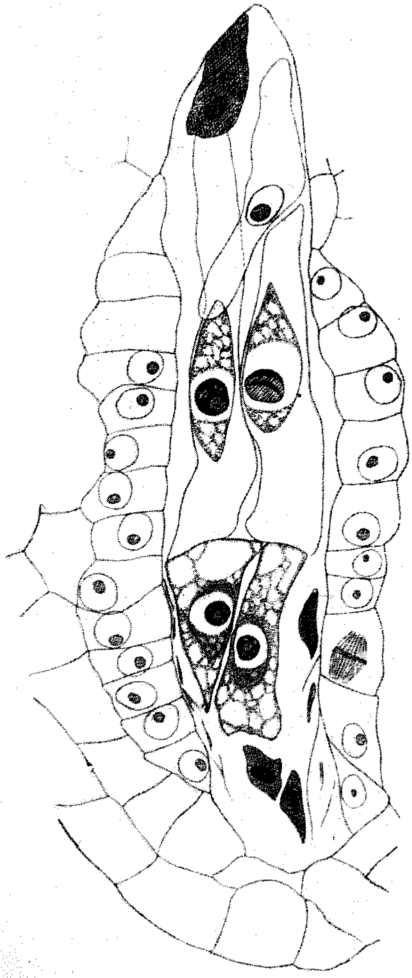


Fig. 8.
738 ×

In another case (very rare in preparations) three cells could be noticed at the chalazal end of the embryo-sac: two of them were uninucleate and the third binucleate (Text Fig. 7 c).

And finally a very interesting case has been observed (only once in preparations): one cell at the base of the endosperm containing three nuclei in an axial row (Text Fig. 7 b).

We may conclude from these observations that, in regard of the endosperm formation, there is a very important difference between *Haberlea* and all other up to date studied genera of Gesneriaceae. They can be summarized and compared as follows:

1. *Klugia Notoniana.* According to Balicka Iwanowska (1) the first division is transverse resulting in the formation of two chambers. The ensuing cell division occurs only in the upper chamber. The nucleus in the chalazal chamber divides, but it is not accompanied by cell wall formation; the basal cell of endosperm is large and binucleate.

2. *Klugia zeylanica.* Schnarf (17) examined this species and found that the first division is transverse; the second takes place only in the upper chamber, and is again transverse. The chalazal cell remains undivided and uninucleate.

3. *Ramondia Nathaliae* and *serbica.* Glišić (8) investigated both species of Balcan Ramondiae and found the same condition as already did Schnarf for *Klugia zeylanica*: the first and second division are transverse (the second affects only the upper chamber); the chalazal cell remains undivided and uninucleate till the end of the endosperm formation.

4. *Corytoloma cyclophyllum.* According to V. Laurent (13) the first division is transverse, the second takes place in the upper chamber and is longitudinal. In the lower chamber only the nucleus divides, thus resulting in the formation of a binucleate chalazal cell.

5. *Roettlera.* My investigations (still unpublished) of this species showed the same condition as given for *Corytoloma.*

6. *Haberlea rhodopensis.* As could be understood from description given in the present paper, this plant does behave in the most of cases in the same manner as *Corytoloma* and *Roettlera:* the first division is transverse, the second occurs in the upper chamber and is longitudinal. At the base of endosperm is to be seen only one binucleate cell. But this is not the only mode. The nucleus division is often accompanied by the cell wall formation, thus resulting in the formation of two uninucleate cells at the chalazal end of the embryo-sac. More than two cells have been observed at the base of endosperm several times. And finally one three-nucleate cell has been found once to occupy the chalazal end of endosperm.

7. *Rhytidophyllum crenulatum.* Cook (3) has described only the cellular formation of endosperm in this plant. A large pearlike cell is occypying the chalazal end of the embryo-sac, and probably originates from the first transverse division.

8. *Streptocarpus polyanthus.* Hielscher (10) briefly stated only that the embryo-sac divides into 2, 4, 8, 16 cells, and so a transitory endosperm tissue is formed.

There is no doubt that the genus *Haberlea* represents an old stage in the phylogentic evolution of Gesneriaceae. We have many reasons to be convinced that the cellular stage must be older and more primitive than the free nuclear (Schürhoff, 18).

„Even when the endosperm begins with free nuclear division, a rudimentary plate often appears, suggesting derivation from an endosperm in which nuclear division was followed by cell formation". (Coulter and Chamberlain (4) p. 172.). On the other hand a reduction in the number of organs means derivation and represents a progressive stage. *Haberlea* shows a strong tendency to reduce the number of cells at the chalazal end of the endosperm tissue. From the multicellular stage corresponding with some genera of Scrophulariaceae, Labiatae, and others, *Haberlea* has reached, by the process of reduction, the binucleate one-celled stage, *which has to be regarded as its normal present stage.* During this historical process of reduction it was the cell wall which disappeared first, i. e. it failed to form. The cases of partial reduction, met in preparations, may represent the stages towards elimination, thus showing us the true line of evolution pursued. The two-celled or three-celled stages may be understood as reminiscence of a much more remote ancestry with cellular endosperm.

Leaning upon these data we are allowed to suppose that the next step in the reduction will affect once more the chalazal binucleate cell, i. e. its number of nuclei. The number of nuclei will probably be reduced to 1. And so *Haberlea* in the next future will attain the stage at which are presently found to be *Ramondia Nathaliae, R. serbica* and *Klugia zeylanica* among Gesneriaceae. The observed stages in *Haberlea* form a real sequence. *Haberlea* shows even a more primitive stage than *Corytoloma* and *Roettlera,* since in both last genera the number of nuclei in the chalazal cell is already fixed to 2, whilst this number is mostly reached but not yet stabilized in *Haberlea.* There is no doubt that *Ramondia* and *Klugia zeylanica* represent the final stage in the phylogenetic evolution of Gesneriaceae, which are investigated up to date. They are, in this respect, specialized rather than primitive forms.

It may be of interest to call attention here to Fritsch's (7) and Hayek's (9) recent investigations relating to the morphology and mode of branching of the inflorescence in *Ramondia Myconi* (7) and *Jankaea Heldreichii* (9). Hayek compares the inflorescence of these plants with that of *Haberlea rhodopensis* and concludes that the latter species, in this respect, is representing a more primitive type, on account of still retaining the bracts and fore-leaves and dichasial branching of the inflorescence.

These primitive morphological characters are in accord with those of the endosperm (as described in the present paper) and are strongly supporting the opinion on the primitiveness of *Haberlea.*

The liability to degradation and disappearance shown by the chalazal cells is probably to be correlated with a capability for a rapid evolution; and, in case of *Ramondia,* with its accomodation to the new post-tertiary life conditions. The recent experiments of Košanin upon both Balcan *Ramondia*-species, especially *R. Nathaliae* (still unpublished) are of great importance showing us clearly that this plant is exceedingly plastic. In regard to large changes of temperature and extreme dryness this plant is highly resistent and accomodated. It can be dessicated to a measure of dryness that equals that of the seed, containing 12—14% of water. The experiments have also shown that the plant in such a dry stage does not loose its vitality for a very long time; in herbars have been found plants to be still alive, even after a year of dessication. In his earlier studies on *Ramondia*-species Košanin (11, 12) has shown that *R. Nathaliae* is a plant which acommodates itself to a very great extent to the climatic and edaphic conditions. It has a large height amplitude and can be found beginning from the mediterranean region and up to 2.200 m. in the mountains. The vegetative period of *Ramondia,* under its life conditions, is very short. Therefore, the development of this plant is forced.

We must recognize that there are still many difficulties in extending this interpretation to all cases observed; numerous facts are still needed, and we must await the results of investigation of many other genera of Gesneriaceae before we access the generalization and conclusion of general importance.

The development of the embryo could not be traced on account of the lack of material. The oospore is not attached to the very tip of the embryo-sac, but a little beneath it, laterally (Pl. II, Fig. 4.). That was also the place of insertion of the egg cell. After the fecundation the oospore elongates very rapidly into a long oospore tube, whose distal end is somewhat swollen bearing nearly the whole protoplasmic contents. The oospore tube is finding its way between the endosperm cells, and is ascending as far as the basal cells of the central endosperm tissue (Text Fig. 5.).

In connection with the recent paper of Dahlgren (5), dealing with the question of apparition of the starch grains in the embryo-sac, I could add one plant more of Gesneriaceae whose embryo-sacs do contain such grains. The starch grains are to be found very abundantly in the micropylar region of the ovule and in placenta at every stage of development. These grains behave chemically differently towards the iodine; those in placenta stain blue and those in the region of micropyle reddish. Very young embryo-sacs do not show any trace of this substance. When the embryo-sac reaches the eight-nucleate stage and later in the endosperm cells it is common to observe the starch grains, which stain also reddish. They are smaller in the sac than those in the micropylar region or in the placenta.

(From the Botanical Institute, Philos. Faculty, University of Beograd)

LITERATURE CITED:

1. *Balicka-Iwanowska, G.:* Contribution à l'étude du sac embryonnaire chez certains Gamopétales. — Flora, Bd. 86., 1889.
2. *Cihlar, C.:* Prilog poznavanju anatomske gradje nekih Cyrtrandoidea. — Rad Jugosl. Akad. Znanosti i Umjetnosti, mat.-prirod. razred, vol. 195., 1913.
3. *Cook, M. Th.:* The embryology of Rhytidophyllum crenulatum. — Bull. Torrey Bot. Club, Vol. 34., 1907.
4. *Coulter and Chamberlain:* Morphology of Angiosperms. — New York, 1909.
5. *Dahlgren, K. V. O.:* Über das Vorkommen von Stärke in den Embryosäcken der Angiospermen. — Ber. d. d. Bot. Gesell., Bd. 45., 1927.
6. ———— : Die Morphologie des Nuzellus mit besond. Berücks. der deckzellosen Typen. — Jahrb. f. wiss. Bot., Bd. 67., 1927.
7. *Fritsch, K.:* Der Blütenstand von Ramondia Myconi. — Ber. d. d. Bot. Gesell., Bd. 45., 1927.
8. *Glišić, Lj.:* Development of the female X-generation and embryo in Ramondia. — Diss., Beograd, 1924.
9. *Hayek, A. von:* Der Blütenstand von Jankaea Heldreichii. — Magyar Botanikai Lapok, Bd. 25., 1926.
10. *Hielscher, T.:* Anatomie u. Biologie d. Gattung Streptocarpus. — Cohn's Beitr. z. Biol. d. Pflanz., Bd. 3., 1879.
11. *Košanin, N.:* La distribution géographique des deux espèces de Ramondia du Balcan. — Glas Srpske Kralj. Akad., Vol. 101., 1922.
12. ———— : Les plantes de reliquat de l'époque tertiaire dans la flore actuelle. — Ibid., Vol. 107., 1923.
13. *Laurent, V.:* Zur Entwicklungsgeschichte von Corytoloma cyclophyllum. — Svensk Bot. Tidskr., Bd. 17., 1923.
14. *Schmid, E.:* Beiträge zur Entwicklungsgeschichte der Scrophulariaceen. — Beih. z. bot. Centrbl., Bd. 20., Abt. 1., 1906.

15. *Schnarf, K.:* Zur Entwicklungsgeschichte von Plantago media. — Sitzb. Akad. Wiss. Wien, math.-nat. Kl., Abt. 1., Bd. 126., 1917.

16. ———— : Beiträge zur Kenntnis d. Samenentwicklung der Labiaten. — Denkschr. Akad. Wiss. Wien, Bd. 94., 1917.

17. ———— : Kleine Beiträge zur Entwicklungsgeschichte der Angiospermen:

a) . . . II. Klugia zeylanica. — Oesterr. Bot. Zeitschr., Bd. 70., 1921.

b) . . . V. Über zwei kritische Fälle d. Endospermentwicklung (Verbena und Triglochin). — Ibid., Bd. 74., 1925.

18. *Schürhoff, P. N:* Die Zytologie d. Blütenpflanzen. — Stuttgart, 1926.

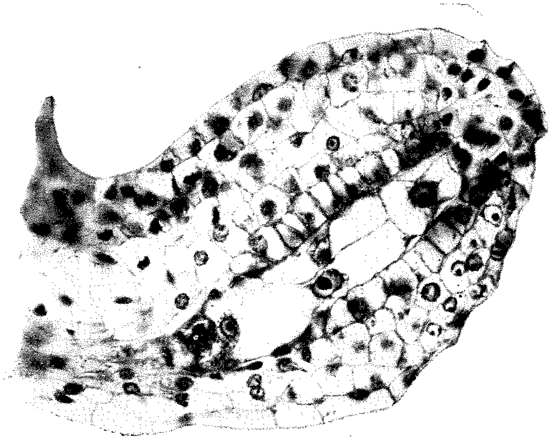
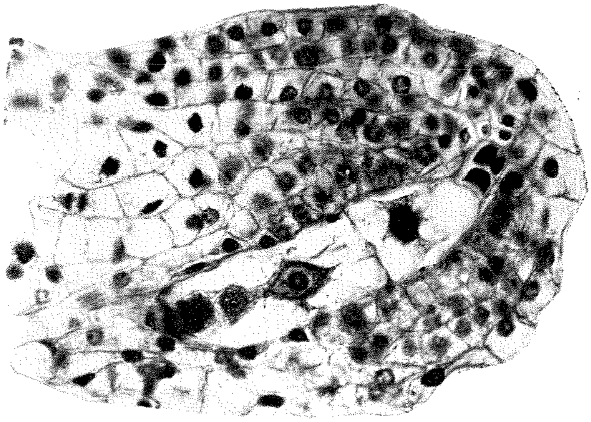


Fig. 3.



2. Fig.

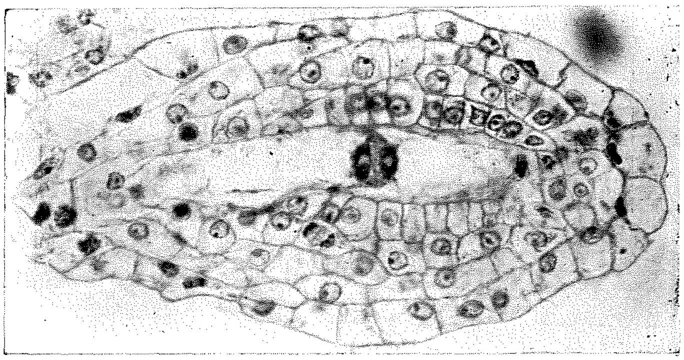


Fig.

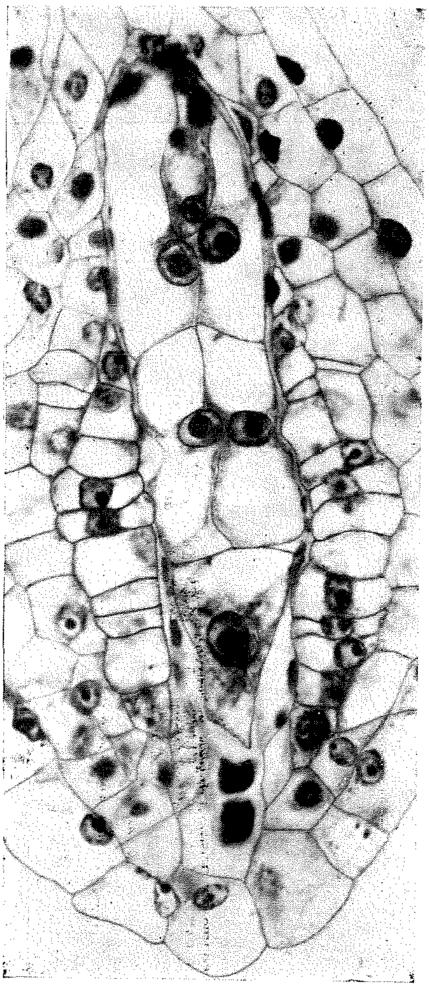


Fig. 4.

Plate I.

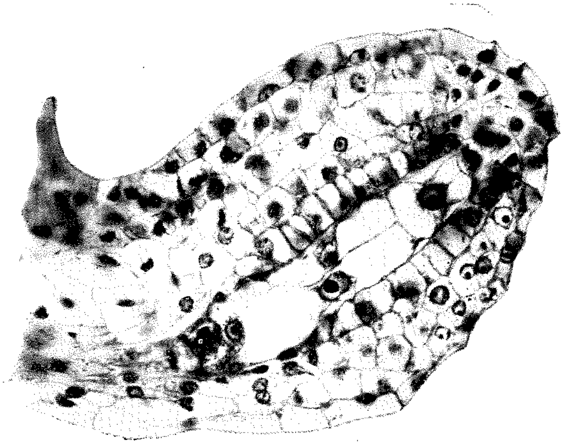
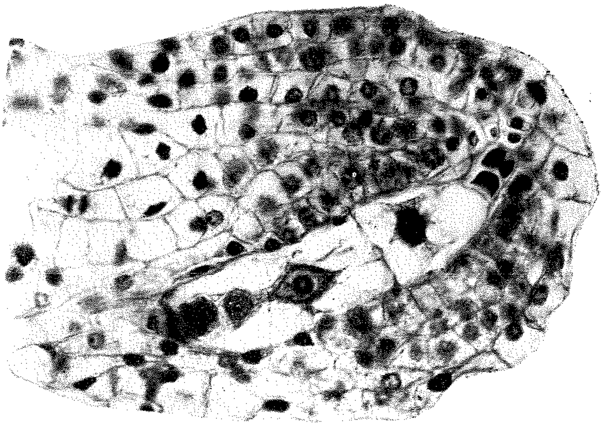


Fig. 3.



2. Fig.

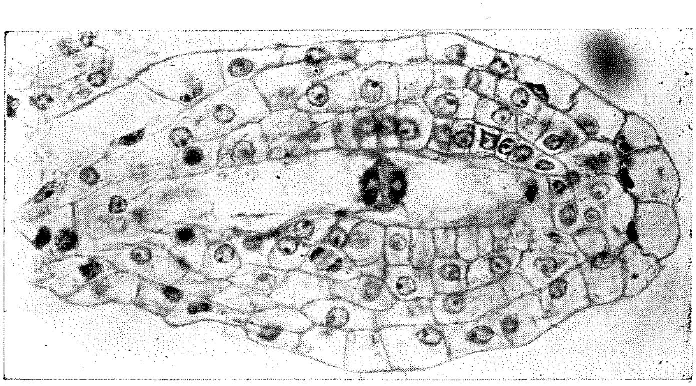


Fig.

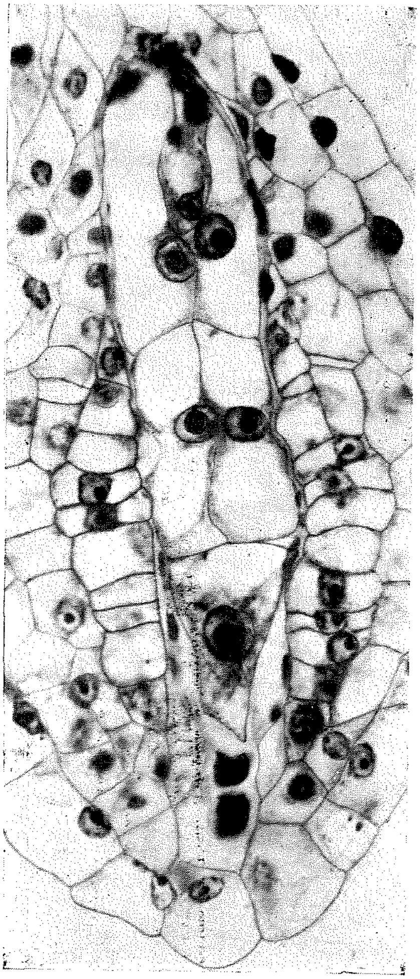


Fig. 4.