

Macrosporogenesis and embryo sac development in *Chondropetalum Hookerianum* (Mast.) Pillans (*Restionaceae*)

Makrosporogeneza i rozwój woreczka zalążkowego u *Chondropetalum*
Hookerianum (Mast.) Pillans (*Restionaceae*)

by

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INTRODUCTION

The family *Restionaceae* is at present very little known embryologically. To the best of the authors knowledge the only papers published on this subject are his own and his co-worker's (Borwein, Coetzee and Krupko (1949), S. Krupko 1962 and F. Młodzianowski in press).

These papers deal with the following species of *Restionaceae*; *Elegia racemosa*, *Restio dodii*, *Hypodiscus aristatus*, *Thamnochortus fruticosus*.

Besides these, caryological investigations by dr. Barbara Briggs (Sydney, Royal Bot. Garden, Australia) are in the train of publication.

Evidently the representatives of only 4 genera have been investigated i.e. *Elegia*, *Restio*, *Hypodiscus* and *Thamnochortus*. This is highly deficient coverage of the family especially if we take into consideration 1) that the genus *Restio* comprises more than one hundred species, growing on two continents — Africa and Australia. This is equal to 1/3 of the number of species included in the whole family, 2) that is so happened that only the older embryo sac stages were collected in the genus *Thamnochortus*. The author now adds *Chondropetalum Hookerianum* to this modest number of species investigated. Both the species and its genus are endemics to South Africa. The genus includes 19 species according to the Pillans monographs (Pillans 1928a, 1928b, 1928c). All of them are dioecious as are nearly all members of the family *Restionaceae*. *Chondropetalum Hookerianum* is quite a frequent plant in the slopes of Table Mountain and other mountains of the Cape Peninsula. Its ovary has 2—3 chambers and bears 3 stigmas. One orthotropous ovule develops in each ovary chamber. Vestigial pistils are present in the flowers of the male plants. In the flowers of female plants staminodia occur only occasionally.

TECHNIQUE

The material i.e. ovaries and male flowers of *Chondropetalum Hookerianum* was collected on the slopes of the mountains in the vicinity of Muizenberg plateau, near Capetown in December 1948 and in January 1949. It is worth noting that the time of flowering is not given in the Pillan's monograph. In Adamson and Salter's book pg. 143, however, he mentions March — April.

The material was fixed in Navashin's fluid (Stockholm modification), in FAA and in Carnoy. The anthers were fixed in Carnoy and in AA.

Imbedding in paraffin was carried out in the Botany Department of Johannesburg University.

The thickness of the sections varied between 8—16 μ according to the developmental stage of the organs being cut. The staining procedures used were: Iron Hematoxylin with Fast Green, Cristal Violet, Feulgen method, McManus method in combination with Iron Hematoxylin. A number of reagents were further used for the detection of suberin, lignin and starch.

RESULTS

ANTHER AND POLLEN GRAIN STRUCTURE

The anthers of *Chondropetalum Hookerianum* have two pollen grain sacks i.e. they are two chambered as are anthers of all members of the *Restionaceae* in South Africa.

The material was found to contain too few young stages for the study of meiosis to prove profitable. It consisted mainly of mature pollen grains. The mature pollen grains were almost invariably 2-nucleate. In contrast to *Hypodiscus aristatus* 3-nucleate pollen grains were exceptionally rare in *Chondropetalum Hookerianum*. After the Feulgen method the tube nucleus was found to be unstained or slightly yellow while the generative nucleus stained a deep red. The generative nucleus was found to be very small, even smaller sometimes than sperm nuclei in *Hypodiscus aristatus*. Mature pollen grains after Hematoxylin or Crystal Violet show both nuclei stained well.

The methods used were inappropriate to enable a decision to be made as to the presence or absence of male cytoplasm around the generative nucleus. The anther wall is 4-layered and very similar in structure to the same in *Hypodiscus aristatus*.

MACROSPOROGENESIS

In the orthotropous ovules of *Chondropetalum Hookerianum* the macrospore mother cell always appears in the subepidermal layer of the nucellus (Fig. 1). At about the same time the external integument reaches

the top of the nucellus while the internal integument is much lower. This developmental sequence differs from that in *Hypodiscus aristatus* where the macrospore mother cell appears in the nucellus while the external integument reaches half the nucellus height and the internal integument reaches only the bottom of the nucellus.

No precise correlation between the developmental stage of the integuments and the time of m.m.c. initiation was established. At the stage described the epidermis of the nucellus doesn't differ from the bulk of the nucellus except for 2—3 cells at the top of it which are more elongated.

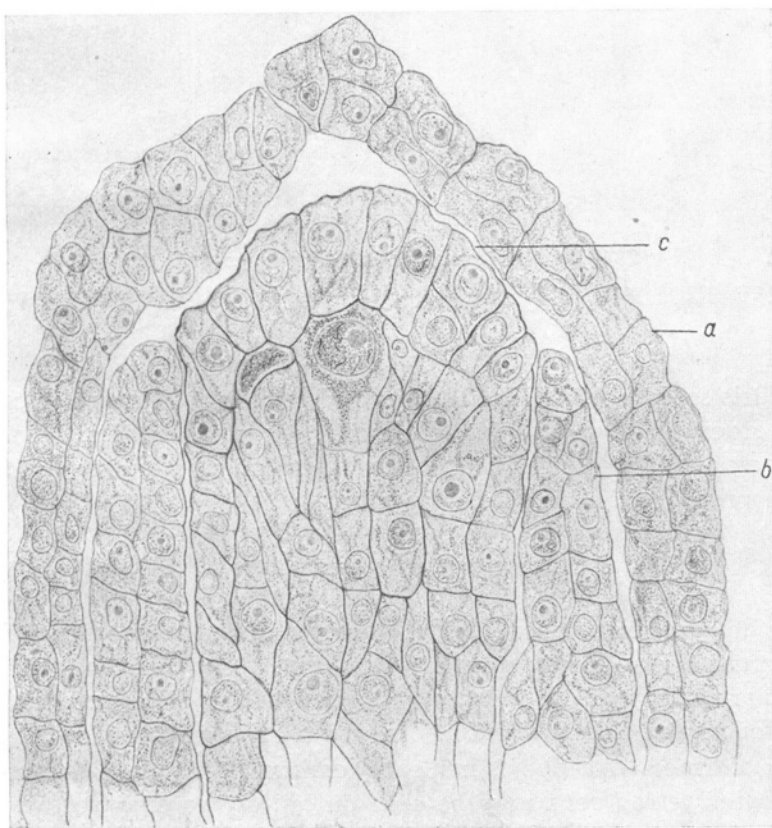


Fig. 1. *Ch. Hookerianum*. Macrospore mother cell

a — outer integument; b — inner integument; c — epidermis

Fixation: Carnoy and chloroform. Thickness: 8 μ . Stained: Feulgen. Magnification: 1065 \times

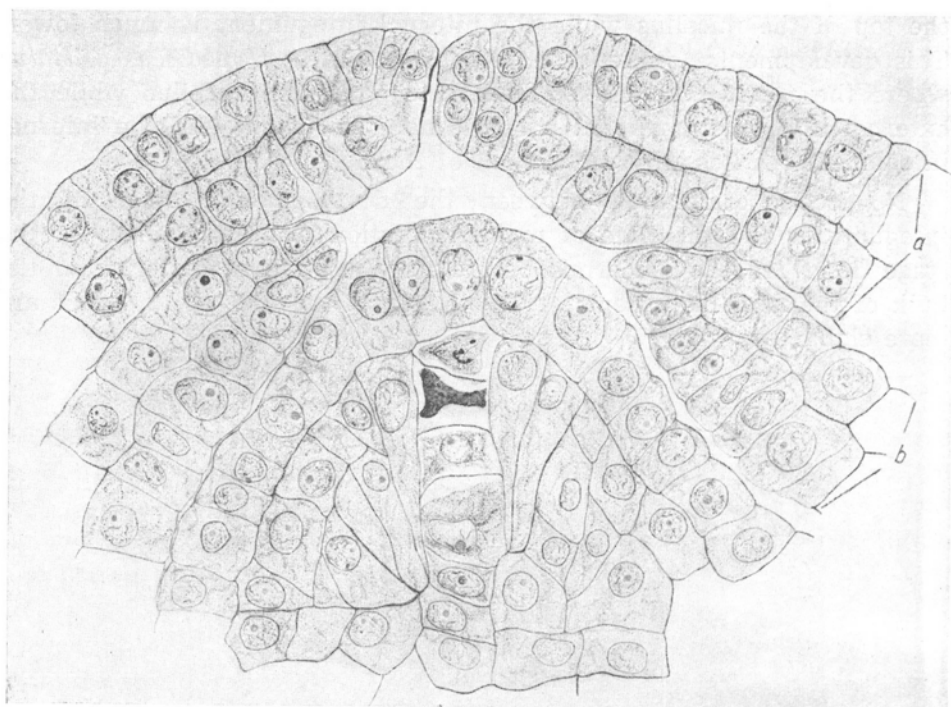


Fig. 2. *Ch. Hookerianum*. Tetrads. Abbreviations as in the fig. 1

Fixation: Nawashin Stock; Thickness: 10 μ ; Stained: Iron Haematoxylin and Fast Green; Magnific.: $\pm 800 \times$

Meiosis soon follows (Fig. 2) diads and tetrads are formed in the usual linear order. At the beginning of micropylar macrospores degeneration the external integument forms the micropyle and the internal integument approaches the top of the nucellus.

EMBRYO SAC DEVELOPMENT

The chalazal macrospore of the linear tetrad of macrospores develops further as a one — nucleate embryo sac (Fig. 3). At this time the nucellar epidermis in *Hypodiscus aristatus* is much better differentiated than in *Chondropetalum Hookerianum*. In both species the micropyle is already formed. At the 2 nucleate embryo sac stage remnants of degenerated tetrad cells may be seen on top of the embryo sac (Fig. 4). The embryo sac is now much grown. As it expands it presses the nearest cells of the nucellus. The cells change their appearance under this pressure and some of them situated next to the embryo sac become very much elongated. At the stage of the 8-nucleate embryo sac these become even more conspicuous. There are, however, 4—6 already differentiated epidermis cells above the 2 nucleate embryo sac.

The four-nucleate embryo sac (Fig. 5) is 2—3 times longer and wider than the 1-nucleate one. Its nuclei are smaller. The nucellar epidermis is better developed. The nucellus cells adjacent to the embryo sac are more compressed and elongated, some of them have started to degenerate.

The embryo sac attains an even larger size at the 8 free nuclei stage (Fig. 6). It is approximately of the shape of an elongated quadrangle in longitudinal section. Nucellus cells along its length are flattened and very elongated. Their nuclei are flattened too, but as yet not degenerating. These cells give such a characteristic appearance to the longitudinal sections through the ovule that they deserve to be called a lining even though the name is temporary as the cells are destined to be finally squashed (Fig. 7).

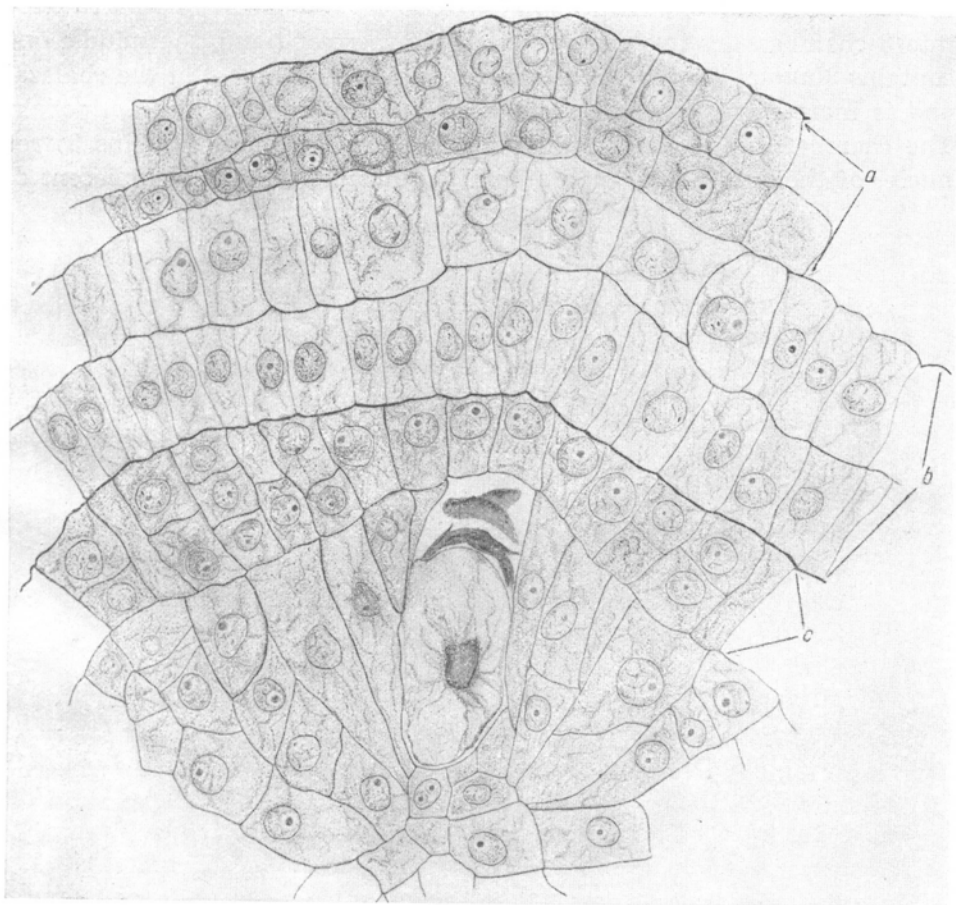


Fig. 3. *Ch. Hookerianum*. One-nucleate embryo-sac. Abbreviations as in Fig. 1
 Fixation: Nawashin Stock; Thickness: 8 μ ; Stained: Iron Haematoxylin and Fast Green; Magnific.: $\pm 800 \times$

Further important changes are noticeable on similar longitudinal sections through the ovule. There is a cushion of small densely crowded cells at the chalazal end which are later destined to be lignified. This is the hypostaze. The internal layer of cells of the inner integument is simultaneously found to have increased in size, its cells have elongated and are found to be filled with tanins. Soon afterwards this layer gives rise to the endothelium with cutinized periclinal walls. They show up well after Sudan III.

The nucellus epidermis has now some 8—10 non-dividing cells, which are very elongated. Four similar slides were obtained of embryo sacs at the 8 free nuclei stage. The fourth of these represented a developmental stage further advanced towards the formation an egg apparatus (Fig. 8).

This was, however, rather different from the conventional description of the developmental sequence. The 8 free nuclei are again visible, but in a different alignment. The embryo sac is here seen to be partitioned into 3 chambers by two crosswalls. The largest of these, the middle one contains 2 nuclei, evidently the polar nuclei. The chamber at the chalazal end is more elongated and has 3 free nuclei, probably the antipodals. The chamber at the micropyle end is rather wider and contains 3 free nuclei of the future egg apparatus. This unusual slide is reminiscent of

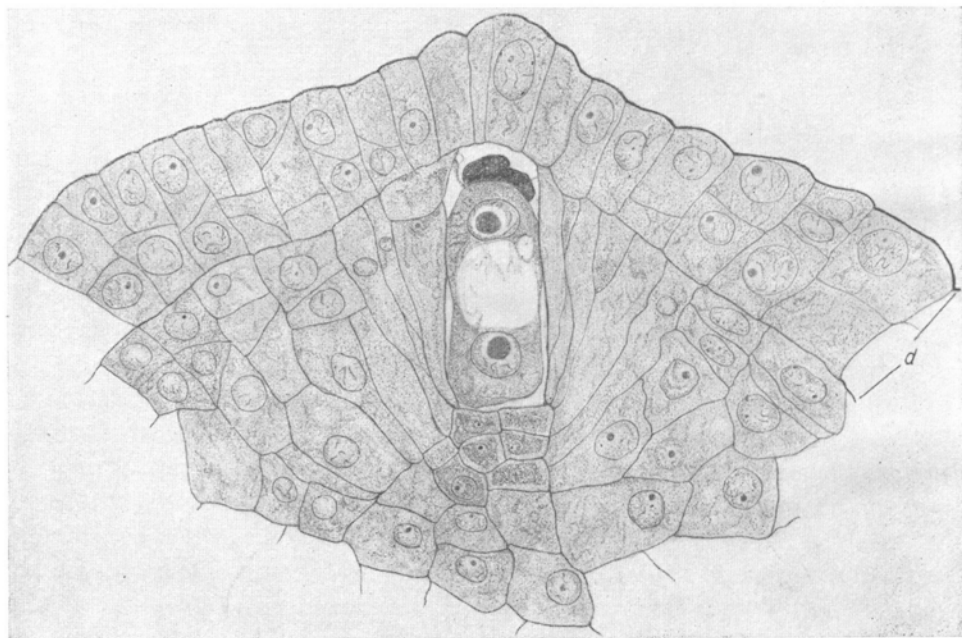


Fig. 4. *Ch. Hookerianum*. Two-nucleate em. s.

d — endothelium

Fixation: Nawashin Stock; Thickness: 10 μ ; Stained: Iron Haenatoxylin and Fast Green; Magnific.: $\pm 800 \times$

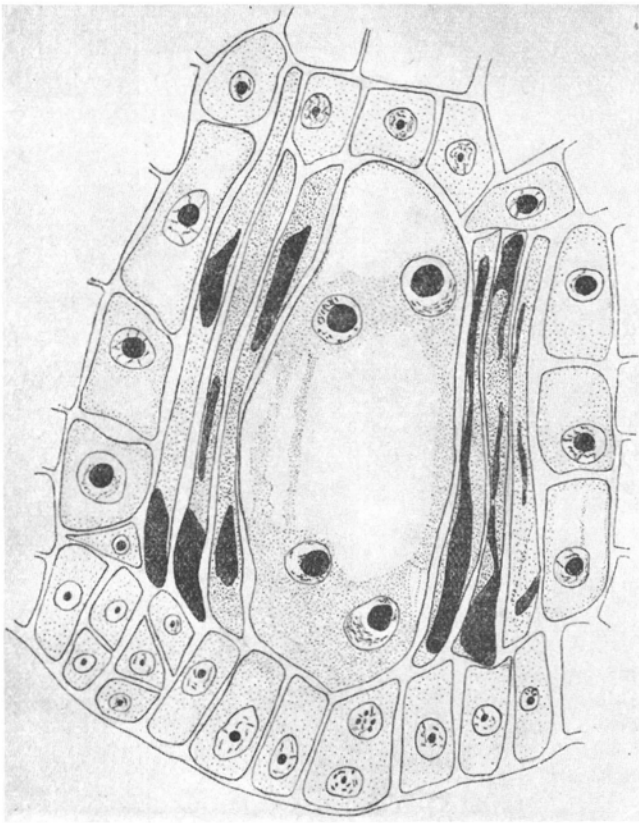


Fig. 5. *Ch. Hookerianum*. Four-nucleate em.-s.

Fixation: Nawshin Stock; Thickness: 8 μ ; Stained: Iron Haematoxylin and Fast Green; Magnific.: $\pm 800 \times$

D. C. Cooper's (1938) picture of a 4-nucleate, 3-chambered embryo sac in an American species of *Zea mays*. The middle chamber in *Zea mays* also had 2 nuclei. This partitioning of the corn's embryo sac occurred at the 4-nucleate stage while in *Chondropetalum Hookerianum* it appears at the 8-free nuclei stage. Of the four slides obtained of this stage the fourth one (Fig. 8) apparently represents a more advanced stage in development. This assumption is borne out by the shape of the embryo sac in longitudinal section. In contrast to the shape of the embryo sac in the 3 other preparations which is that of an elongated quadrangle, the chalazal end is much narrower. Furthermore 2 transverse walls are present.

It is difficult to evolve an interpretation on the basis of a single occurrence. One can only presume that this is the case of some retardation in wall formation around the nuclei of the egg apparatus and the antipodals.

The mature embryo sac with 8 nuclei and 7 cells is represented in

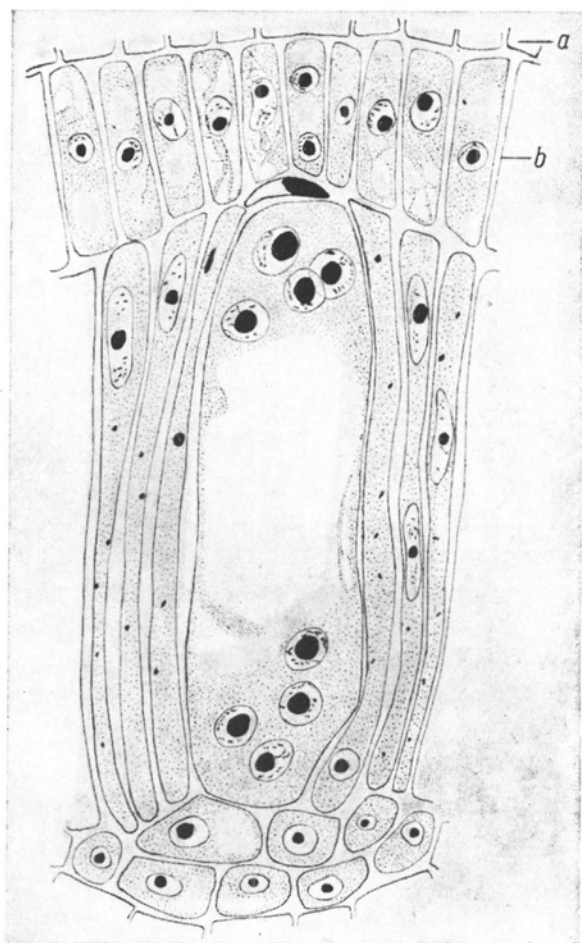


Fig. 6. *Ch. Hookerianum*. Eight-free nuclei stage of em.-s.

a — inner integument; b — epidermis

Fixation: Nawashin Stock; Thickness: 8 μ ; Stained: Iron Haematoxylin and Fast Green; Magnific.: $\pm 800 \times$

Fig. 9. Its micropylar part is wider and its chalazal part is narrower. The synergids exhibit a tendency to further growth. In later stages they are transformed into large pearshape cells, their upper parts are elongated and stalk-like. The 3 antipodals are quite conspicuous at this stage but in contrast to the synergids they soon disintegrate. At syngamy they are reduced to small barely visible cushions. The synergid's behaviour is quite different when fertilization is retarded they develop further into large organs filled with reserve substances. They develop some hook-like appendages with age. Their stalk-like upper parts exhibit the presence of cellulose. (Cellulose was demonstrated by the McManus method and by staining with Fast Green Fig. 17.) According to Schnarf's classification (1929) they can be related to the secretion

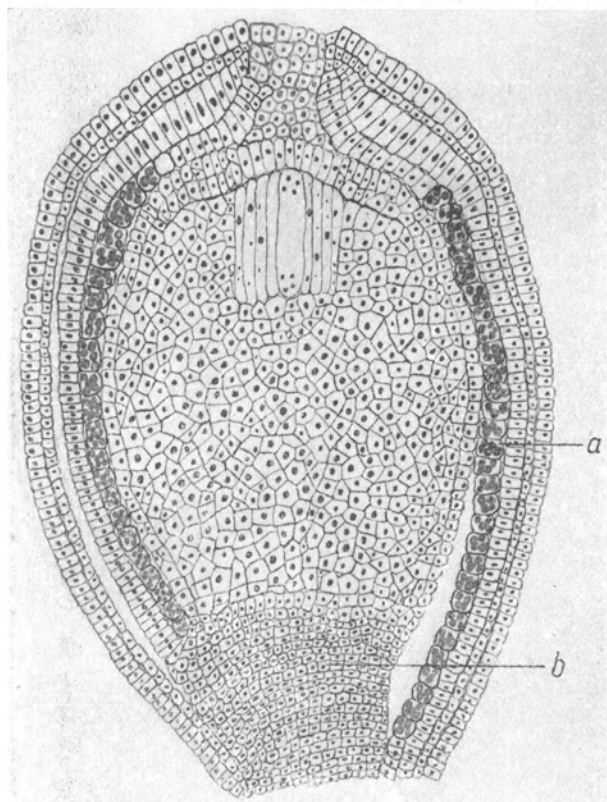


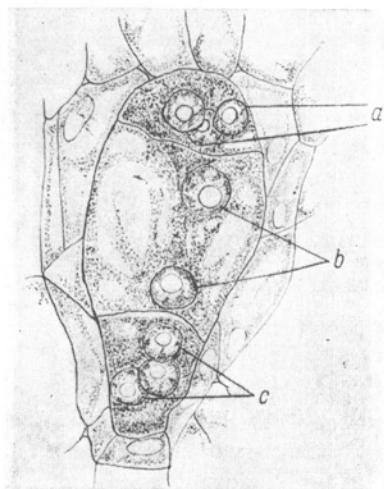
Fig. 7. *Ch. Hookerianum*. The same, showing hypostaze, endothelium

a — endothelium; *b* — hypostaze

Fixation: Nawashin Stock; Thickness: 8μ ;
Stained: Iron Haematoxylin and Fast
Green; Magnification: $\pm 600 \times$

Fig. 8. *Ch. Hookerianum*. Three chambers in 8-free nuclei stage of em.-s

a — egg apparatus nuclei; *b* — polar nuclei; *c* — antipodals nuclei
Fixation: Nawashin Stock; Thickness: 8μ ; Stained: Iron Haematoxylin
and Fast Green; Magnification: $1065 \times$



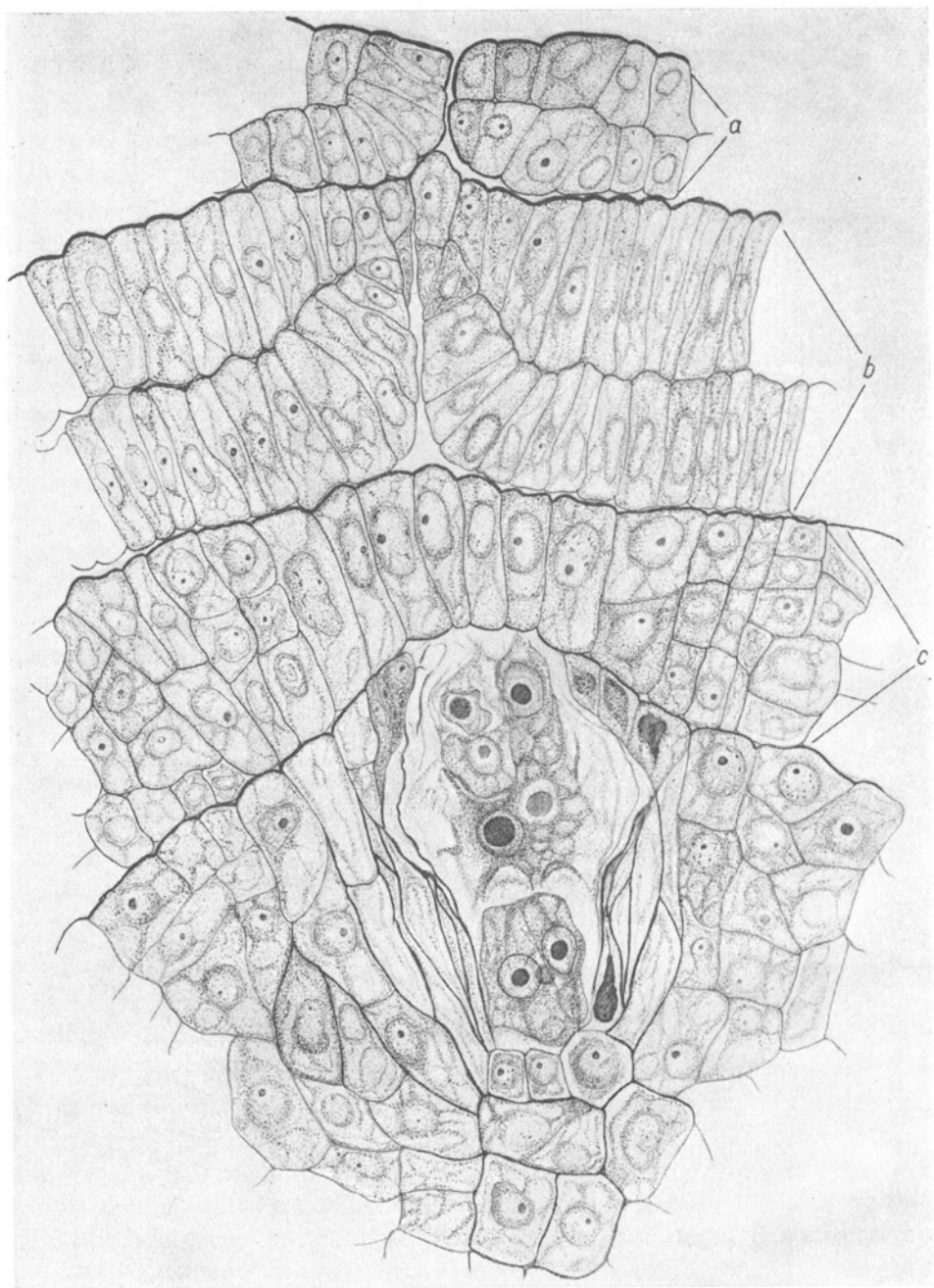


Fig. 9. *Ch. Hookerianum*. Mature em.-s. Abbreviations as in the Fig. 1

Fixation: Nawashin Stock; Thickness: 10 μ ; Stained: Iron Haematoxylin and Fast Green; Magnification: $\pm 1065 \times$

type of synergids. At this stage the nucellar epidermis cells, situated just above the embryo sac enlarge and elongate and their external walls thicken. The cells directly above the embryo sac are not active, but those along both sides of the nucellus frequently divide periclinally. Many nucellus cells along both sides of the embryo sac are already squashed.

SYNGAMY AND ENDOSPERM DEVELOPMENT

Double fertilization was observed in a few cases but it was frequently connected with some form of retardation of the process or with some symptoms of degeneration.

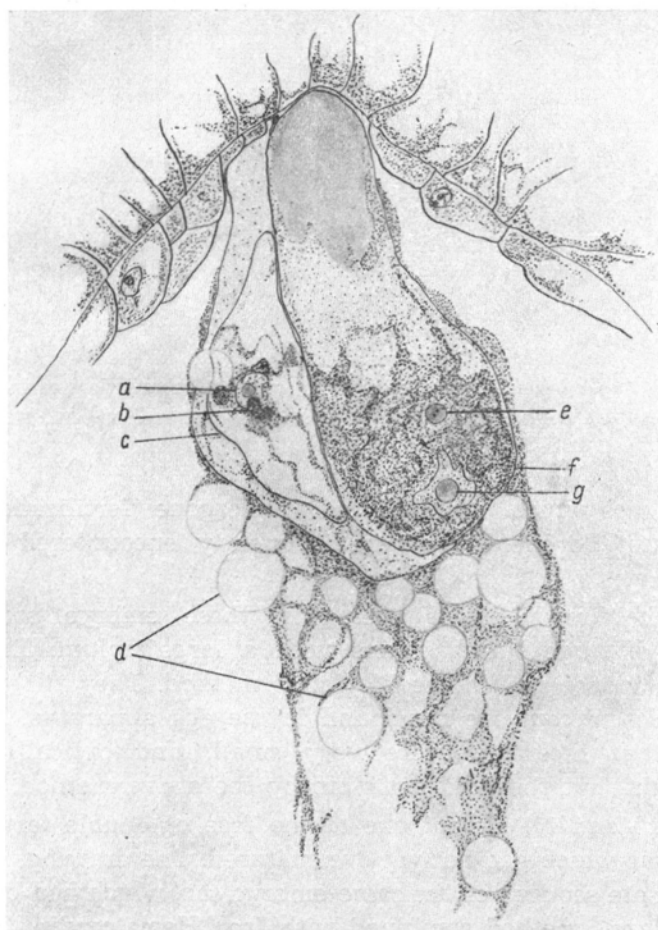


Fig. 10. *Ch. Hookerianum*. Double fertilization

a — nucleus of egg cell; *b* — sperm nucleus; *c* — egg cell; *d* — starch grains; *e* — tube nucleus; *f* — synergid cell;
g — synergid nucleus

Fixation: F. A. A. Thickness 14 μ ; Stained: McManus and Haematox. Magnific.: $\pm 1065 \times$

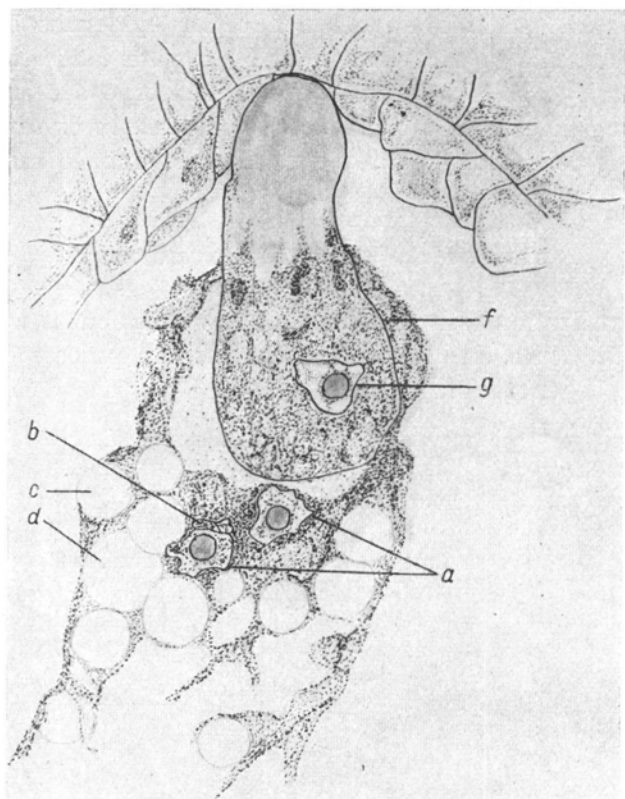


Fig. 11. *Ch. Hookerianum*. Double fertilization (contin.) Abbreviations as in the fig. 10

Fixation: F. A. A.; Thickness: 14 μ ; Stained: McManus and Hematox.; Magnific.: 1065 \times

Embryo sacs which had still not undergone fertilization but had already begun degenerating were frequently encountered in already lignified ovaries.

One of the first signs of ageing in these embryo sacs was the appearance of huge starch grains with a large hylum. These starch grains stain a deep red in the McManus method. They fill the embryo sac when present, but are not found in the egg apparatus. Any starch grains found in the latter were very small, and optically active in polarized light, but they did not stain by the above method.

Figures 10 and 11 present one of the case of double fertilization in an embryo sac which had not yet overmatured. The drawings were based on two separate sections of the same embryo sac, which had been stained by the McManus method combined with Iron Hematoxylin. The outline of the egg cell and its nucleus are visible on the left of Fig. 10. A large part of the egg cell is covered by the huge synergid best seen on the right of the drawing. It is filled with reserve substances.

The sperm nucleus may be seen lying next to the egg nucleus, but below it and to the right of it. It stains deeper with Iron Hematoxylin than the egg nucleus. A little above the egg nucleus and to the left of it are two more, indistinct small bodies which stain strongly with Iron Hematoxylin. They may be the remains of the pollen grain tube? Surprisingly enough there are no distinct pollen grain tube remains in this slide. Perhaps the outline of a membrane below the egg cell wall and to the left of it may be regarded as a pollen grain tube wall. This would be feasible if the pollen grain tube entered the embryo sac bypassing both synergids. The second synergid as conspicuous as the first is presented in Fig. 11.

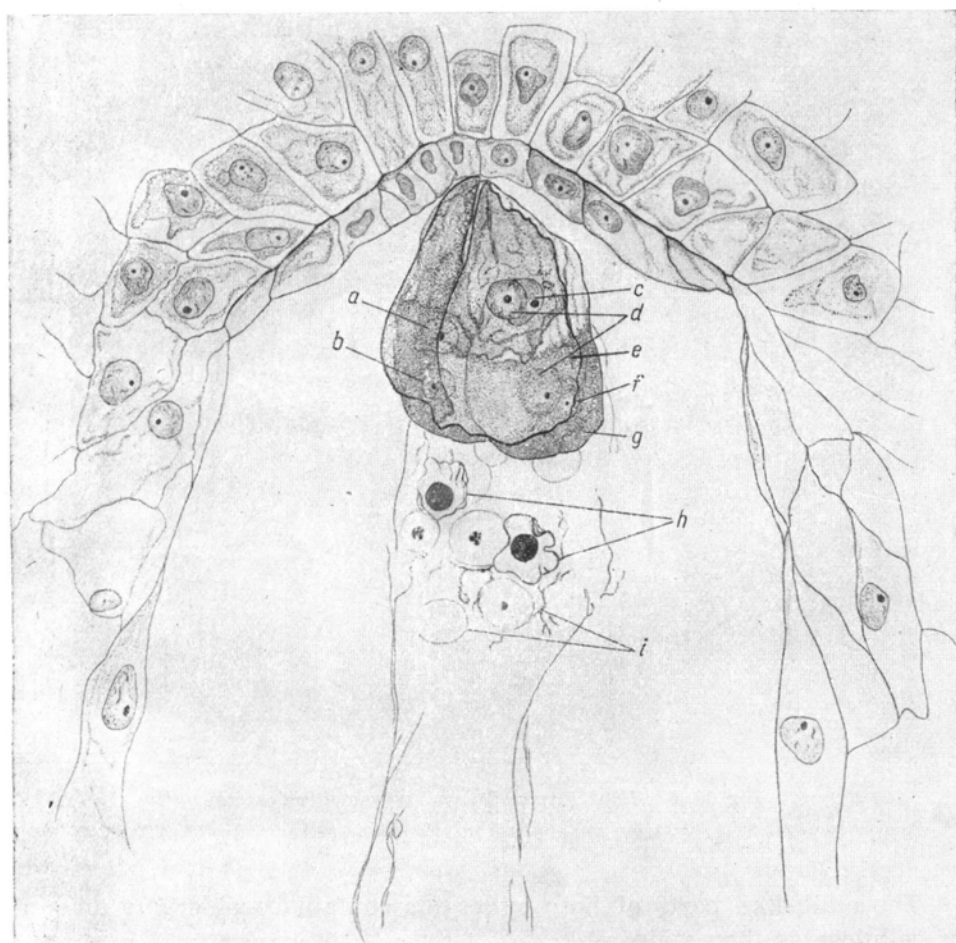


Fig. 12. *Ch. Hookerianum*. Retardation of fertilization

a — Tube nucleus; *b* — synergid cell; *c* — nucleus of egg cell; *d* — sperm nuclei; *e* — egg cell; *f* — synergid nucleus; *g* — synergid cell; *h* — polar nuclei; *i* — starch grains

Fixation F. A. A.; Thickness: 14 μ ; Stained: McManus and Haematox.; Magnific.: $\pm 800 \times$

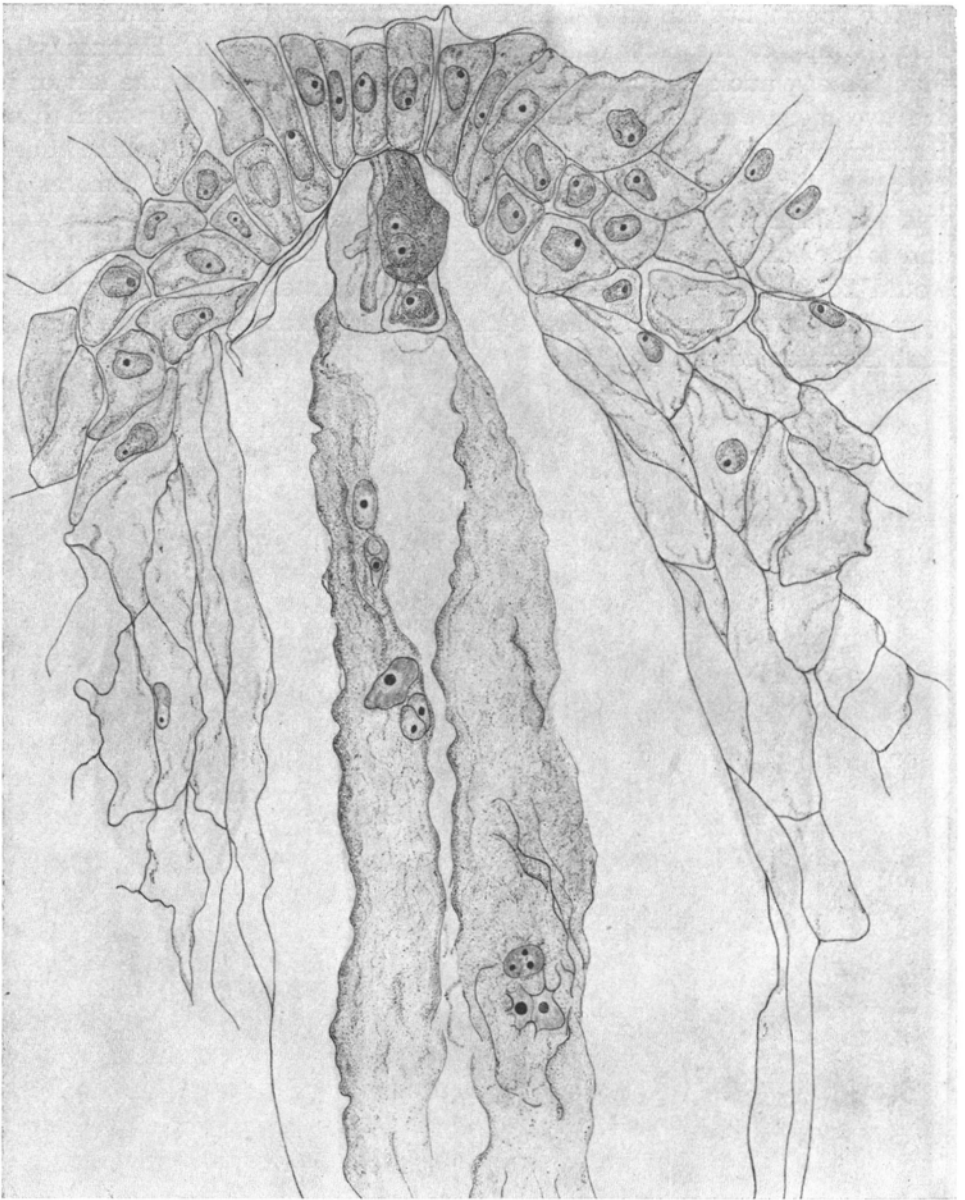


Fig. 13. *Ch. Hookerianum*. Delayed syngamy. Endosperm

Fixation: Nawashin Stock; Thickness: 14 μ ; Stained: Iron Haematox. and Fast Green; Magnific.: $\pm 800 \times$

The stalk-like parts of both synergids contain considerable amount of cellulose as they stain pink.

The two polar nuclei are separated and can be seen in Fig. 11 just below the egg apparatus. The second sperm nucleus may be seen adhering to one of them. Some chromatin threads inside it are visible.

The above drawings show that in this case double fertilization is atypical. In the first place there are no distinct pollen grain tube remains. This indicates a big lapse of time since fertilization. Unfinished syngamy and unfinished fusion between one polar nucleus and the sperm contradict the above supposition. There is a further apparent contradiction in the fact that the polar nuclei are separate and lie at a considerable distance from one another. Yet in *Chondropetalum Hookerianum* this can not be taken as a sign of age since many unfertilized embryo sacs may be found in which degeneration is advanced but the polar nuclei have not fused.

The shape of the polar nuclei on this slide is remarkable. Their circumference shows many invaginations which is a feature of embryo sac degeneration.

All the above features indicate some retardation in the double fertilization process.

Retarded fertilization is even clearer in Fig. 12. This compound drawing shows two synergids which are still in the process of full development and activity. The egg cell is lying on top of them. The outline of its wall is very distinct. The staining procedure used for this slide was the same as for the above one. The sperm nucleus may be seen to the left of the egg cell nucleus and adhering to it. The outline of the pollen grain tube wall forms the background to the left synergid and is situated to the left of the egg cell. Two more nuclei show through this; one is probably the tube nucleus, the other the nucleus of the left synergid.

The second sperm nucleus is presumably the structure lying on the right of the egg apparatus, partially overlapping the right synergid nucleus.

Its progress towards the polar nuclei has been retarded which is probably the reason for its distinct increase in size and for the assumption of the shape of a resting nucleus. The same resting condition is seen in sperm nucleus contacting egg nucleus.

The shape and position of the polar nuclei provides further evidence that the fertilization process has been arrested. These nuclei are placed some distance from one another. Their outer membranes are full of invaginations and convolutions. These are especially prominent in the right nucleus. There are many large starch grains with a large hilum lying in the vicinity of these nuclei. These are usual in this species at the time of degeneration.

In *Chondropetalum Hondropetalum* evidence of endosperm formation is infrequently encountered. Nuclear endosperm develops quite quickly, while syngamy is still incomplete in the young zygote. This is well illustrated in Fig. 13 which was drawn from 3 sections. It shows many

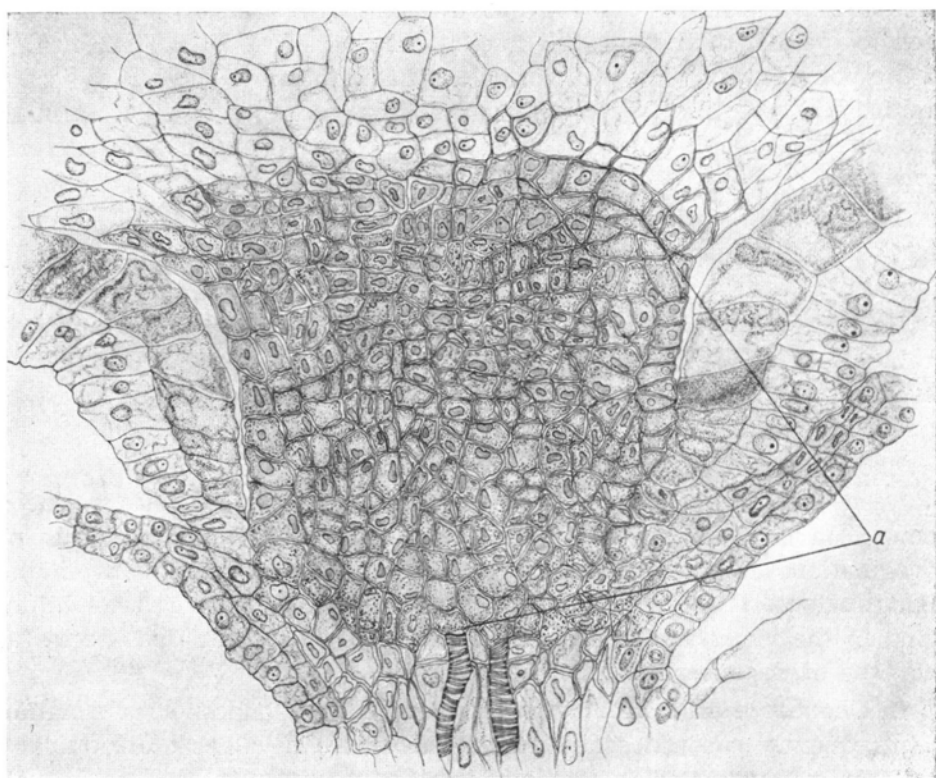
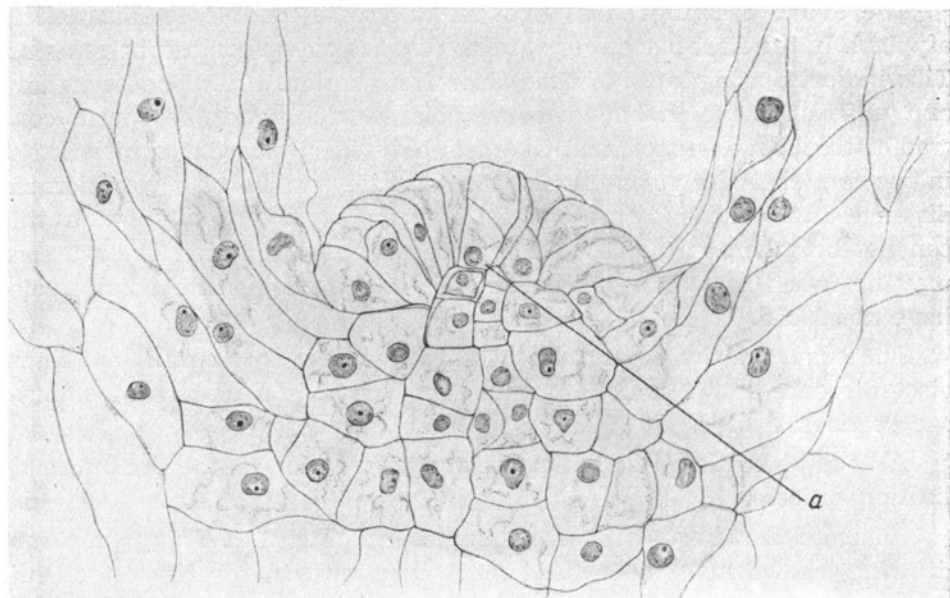


Fig. 14, 15. *Ch. Hookerianum*. Hypostaze and postamentum.

a — postament

Stained: McManus and Haematox.; Magnific.: $\pm 475 \times$

Fixation: F. A. A.; Thickness: 14μ ;

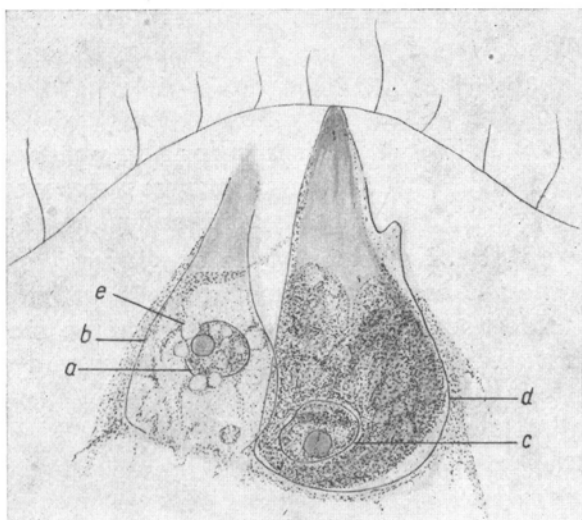


Fig. 16. *Ch. Hookerianum*. Inhibition of fertilization. Persisting synergids

a — nucleus of egg cell; b — synergid cell; c — synergid nucleus; d — synergid cell; e — starch grains
Fixation: F. A. A.; Thickness: 14 μ ; Stained: McManus an Haematox.; Magnific.: $\pm 1065 \times$

endosperm nuclei, the remnants of the left synergid and pollen grain tube and in the egg cell still two nuclei in close contact. The sperm nucleus is here as big as the egg nucleus. In fact it is difficult to distinguish which is which. On the 3 sections, 13 endosperm nuclei were countered. The huge starch grains which are a common feature of unfertilized, over matured or degenerating embryo sacs are absent from the cytoplasm of this one. In the embryo sac just described the right synergid is longer than the egg cell. It is still well preserved. The left one which has been completely destroyed by the passage of the pollen grain tube is empty, with only the cell membrane left.

Further sections of the same embryo sac contain a multicellular cushion-like outgrowth on the bottom. This structure fits the descriptions and illustrations of Dahlgren's (1940) postament very well. This is shown in Fig. 14. for which the drawings were prepared from another slide and embryo sac. The postament is a typical feature of all older ovules in *Chondropetalum Hookerianum*. It was not found in other embryologically described species of the *Restionaceae*. Its presence here is not connected with fertilization at all. It appears in degenerating embryo sacs as well. It is formed after the disintegration of the anti-podals and in about the same position. There are no lignified or suberized cells in this tissue.

At this time the hypostaze (Fig. 15) reaches full development. All

its cells become lignified. This was tested with benzidine in acetic acid, which stains all such cells yellow. After Sudan III only some middle lamellae occasionally stain red. The rare occurrence of ovules with an endosperm and the frequent degeneration of embryo sacs in young fruits in *Chondropetalum Hookerianum* is a surprising phenomenon. More or less advanced degeneration of embryo sacs was recorded in 153 slides out of the 238 prepared from older ovaries and young fruits.

The persistence of the secretory type of synergid is a very typical feature in this species, as already mentioned. It is the organ which is most resistant to degeneration in unfertilized embryo sacs. The egg cell disintegrates first (Fig. 16), the polar nuclei follow next and finally the synergids.

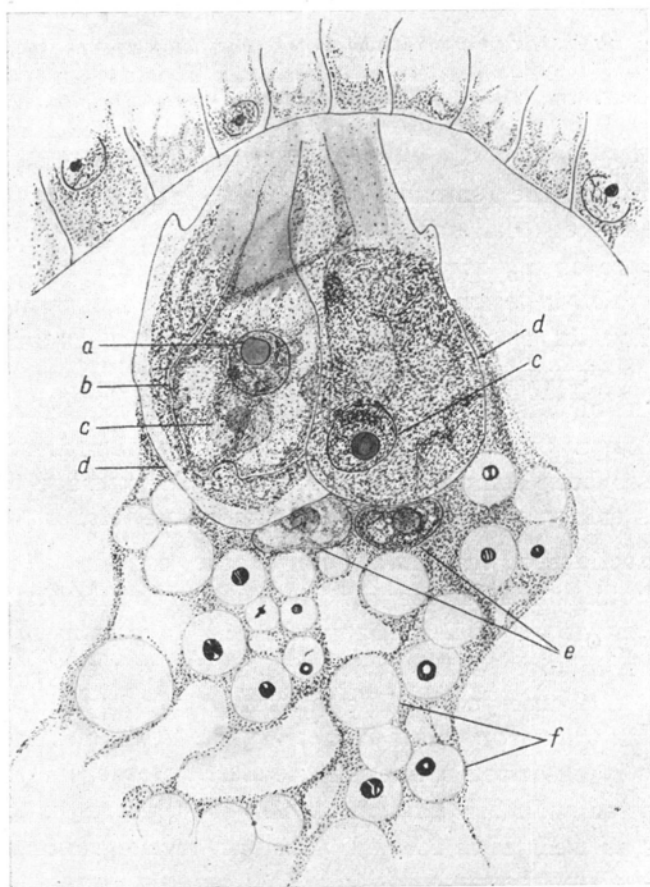


Fig. 17. *Ch. Hookerianum*. The same (contin.)

a — nucleus of egg cell; b — egg cell; c — synergid nucleus; d — synergid cell; e — polar nuclei; f — starch grains.
Magnific.: $\pm 1065 \times$

DISCUSSION

One can distinguish two lines of development among the species of *Restionaceae* investigated on the basis of embryological criteria.

One has embryological features similar to the *Gramineae*. Here belong *Elegia racemosa*, *Restio Dodii*, *Thamnochortus fruticosus* with a tendency, similar to that in the *Gramineae*, towards the multiplication of antipodals.

The other one shows affinities with the *Liliiflorae* and includes *Hypodiscus aristatus* and *Chondropetalum Hookerianum*. The former has 3-nucleate pollen grains, three antipodals which disappear and orthotropous ovules with a short anathropous stage. The latter has 2-nucleate pollen grains and early disappearing antipodals. Of the above two species *Hypodiscus aristatus* is closer to the *Juncaceae* family, *Chondropetalum Hookerianum* is closer to other members of the *Liliiflorae*.

In the *Chondropetalum Hookerianum* material collected in the season in question there wasn't a single ovary or seed found which contained an embryo. There were a few with endosperm and incomplete syngamy. It is difficult to draw conclusions about the frequency of seed sterility in this species on the basis of one season's material.

The time of flowering is worth noting. In 1948—49 young embryo sacs were already available at the beginning of December. They were overmature by January. Pillans gives March and April as the flowering time in Adamson's book (1950).

It is quite possible that Pillans' observations were based on material collected at high sea-level localities in the mountains, at much higher altitudes than this author's sites of collection. This might account for the latter flowering dates. The 1948/1949 season might always have been exceptional in its early flowering time which would explain the large numbers of empty undeveloped seeds with degenerate embryo sacs.

The persistent secretory synergids were not observed in the other 4 members of the *Restionaceae* family which were embryologically investigated. The appearance of the postament in the older embryo sacs is the second unusual feature found in *Chondropetalum Hookerianum*.

SUMMARY AND CONCLUSIONS

1. Mature pollen grains in *Chondropetalum Hookerianum* are 2-nucleate.

2. *Chondropetalum Hookerianum* has orthotropous, crassinucellate, bitegmic ovules with the hypostaze lignified in older stages.

3. The embryo sac is of the *Polygonum* type, with three early disappearing antipodals and with persistent secretory synergids.

4. After desintegration of antipodes in the older embryo sacs the postament is formed.

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STRESZCZENIE WYNIKÓW

1. Dojrzałe pyłki mają dwa jądra.

2. *Chondropetalum Hookerianum* ma ortotropowe zalążki, gruboosrodkowe, o dwóch integumentach, z hypostazą drewniejącą w późnych stadiach.

3. Woreczek zalążkowy należy do typu *Polygonum*, z trzema wcześniej zanikającymi antypodami i z synergidami trwałymi, typu wydzielniczego.

4. Po zniknięciu antypod w starszych stadiach woreczków rozwija się postament.

Praca została wykonana całkowicie dzięki pomocy finansowej Komitetu Botanicznego PAN, za którą serdeczne podziękowania składa autor.

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