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NOTES ON THE STRUCTURE OF THE EMBRYO-SAC IN SPARGANIUM AND LYSICHITON.

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(WITH PLATE I)

RECENT studies on the structure of the embryo-sac of the angiosperms have made it very evident that more variation exists than was formerly supposed. All forms as yet examined agree in the essential characters of the earlier stages of the embryo-sac, but its later stages show much variation, especially in the character and behavior of the antipodal cells. Other marked deviations from the type structure have been recorded, but as yet these are too few and fragmentary to be of much value in interpreting the homologies of the structures of the embryo-sac. These discoveries, however, render it important that a careful study be made of the simpler types of angiosperms, as it is becoming more and more evident that it is among these that the primitive types are to be sought, and here, if anywhere, the clues to the origin of the angiosperms are likely to be discovered.

The writer has been for some time engaged upon a study of the simpler monocotyledons in the hope of being able to throw some light upon their affinities, which at present are very far from being clearly understood. Especial attention has been given to the study of the embryo-sac and embryo. Some of the results of these studies have already been published,[1] and the present paper is a record of some of the more striking facts, especially in regard to the antipodal cells, brought to light in more recent investigations. It is hoped that it will soon be possible to give a fuller account of these points, as well as a study of the development of the embryo in the forms under consideration.

There is a growing tendency to regard the simpler monocotyledons, *i. e.*, those having either no floral envelopes, or inconspicuous ones, as the more primitive types, instead of assuming, as was formerly done, that they are degenerate forms descended from petaloideous ancestors. The present view is clearly expressed in the arrangement of the orders of monocotyledons in Engler and Prantl's *Natürliche Pflanzenfamilien.*[2] Engler's conclusions are drawn mainly from a study of the flowers, and as yet but little has been done, at least of late years, in the study of the development of these presumably primitive types.

During the past two years I have made somewhat extensive collections of material for these investigations, but as yet only a small part of the material has been worked over, and the results here given are only preliminary to what is hoped will be much more complete, studies which may perhaps help to clear up some of the questions as to the affinities of the lower angiosperms.

SPARGANIUM.

The genus Sparganium is a small one, probably comprising not more than ten species, mostly inhabitants of northern regions, but with representatives in New Zealand and Australia. Sparganium is usually included with Typha in the order Typhaceæ, but Engler has separated it as the type of a special order, Sparganiaceæ, with the Pandanaceæ as its nearest relatives.

Some years ago, I had occasion to examine the structure of

[1] CAMPBELL, A morphological study of Naias and Zannichellia. Proc. Cal. Academy of Sciences III. Bot. 1: 1–61. 1897.

Development of Lilæa. Annals of Botany 12: 1–28. Mr. 1898.

[2] Die natürlichen Pflanzenfamilien II. 1: 183 ff. 1889.

the ovule in *S. eurycarpum* Engelm. and was struck with certain peculiarities which it exhibited, some of which were referred to and figured in my paper on Naias and Zannichellia. Most noticeable was the unusual development of the antipodal cells.

The study of Sparganium was interrupted for various reasons, but in the meantime material of other species was collected and the subject taken up again. During the past summer a good supply of *S. simplex* Huds. was obtained from Tallac, on Lake Tahoe, and through the kindness of Miss Alice Eastwood, a quantity of the Californian species, *S. Greenii* Morong, collected at Lake Merced near San Francisco, was sent me. I had also a small amount of a Japanese species, *S. longifolium* Turcz., collected by me at Akkeshi on the island of Yezo. Of these, *S. simplex* proved the most satisfactory for study and these preliminary notes refer principally to that species.

All the species of Sparganium are monœcious, the heads of pistillate flowers being at the base of the inflorescence. There are usually three or four of these pistillate heads in *S. simplex*. Each pistillate flower in this species consists of a single carpel, containing a solitary ovule, and surrounded by a number of membranaceous floral leaves. In *S. eurycarpum* and *S. Greenii*, the pistil is composed of two carpels.

In making sections of the carpels for the study of the embryo-sac, except in the youngest stages, care must be taken to remove enough of the hard outer tissues to permit the penetration of the fixing and embedding agents. Of the various fixing agents employed, the best results were obtained from the use of saturated solutions of corrosive sublimate or picric acid in alcohol. The chromic acid mixtures proved less satisfactory. A double stain of Bismarck-brown and anilin-safranin gave the best results.

All of my material was too old to make it possible to study the origin of the ovule and the early history of the embryo-sac. The development of the flowers has been studied by Dietz,[3] and from his figures it looks as if in Sparganium, as in most of

[3] Bibliotheca Botanica 5: —. 1887.

the simpler monocotyledons hitherto investigated, the ovule is axial in origin.

The early history of the embryo-sac could not be traced, but as the mature sac, at the time of fertilization, corresponds closely with that of the typical angiosperms it is not likely that there are any marked peculiarities in its early development.

In the mature ovule (*figs. 1, 2*) the broad apex of the embryo-sac is covered by two layers of cells, the outer ones being elongated so that the apex of the nucellus is pointed. The cytoplasm is confined to an extremely thin layer lining the wall, and a small amount surrounding the polar nuclei. The egg-apparatus is relatively small but offers no especial peculiarities. The synergidæ (*fig. 2, s*) are smaller than the egg (*o*), and their cytoplasm is densely granular, whereas the egg appears almost transparent. The nuclei of the synergidæ are small, but distinct, and like that of the egg, they possess a conspicuous nucleolus. The nucleus of the egg is somewhat larger than the nuclei of the synergidæ, but otherwise resembles them.

On examining the antipodal region of the embryo-sac (*fig. 3*) the very small size of the antipodal cells (*ant*) is at once noticeable; indeed, where these have collapsed in the process of embedding, it is almost impossible to see them at all.

The polar nuclei are much larger than the other nuclei of the embryo-sac, and probably always fuse before fertilization is effected. This, however, has not been positively shown. In the specimen shown in *fig. 3*, the fusion of the polar nuclei was complete, and the single large endosperm-nucleus (*en*) was very conspicuous. In the embryo-sac of *S. Greenii*, however (shown in *fig. 4*), the two polar nuclei were clearly distinguishable, although they were in close contact.

The actual fertilization has not yet been observed, and owing to the small size of the nuclei of the pollen-spore and pollen-tube, it is not likely that this will be easy to demonstrate satisfactorily.

As usual, the synergidæ disappear after fertilization, and the egg-cell, now become the one-celled embryo (*fig. 6, em*), can alone be distinguished at the upper end of the embryo-sac. There is little change in the embryo for some time, and it remains undivided until after there is a considerable development of the antipodal cells and endosperm.

An examination of the antipodal region of the recently fertilized embryo-sac (*fig. 7*) reveals a remarkable change in the antipodal cells, which seem to be immediately affected by the act of fertilization. While the embryo-sac itself has not increased appreciably in size, the antipodal cells have now enlarged to several times their original dimensions and present all the appearances of actively growing cells. The nuclei have divided, and in the specimen figured there were already present eight antipodal nuclei. It was difficult to decide whether in all cases the division of the nucleus was accompanied by the formation of a division wall, or whether, as in some other cases of multiplication of the antipodal nuclei, the division of the nucleus occurs without the formation of a cell wall.

The first division of the endosperm-nucleus takes place at about the same time that the first nuclear-division occurs in the antipodal cells. The secondary endosperm-nuclei arrange themselves, in the usual way, in the thin layer of protoplasm lining the wall of the embryo-sac, which now rapidly increases in size. Before any cell-division takes place in the endosperm, the antipodal cells have increased enormously in size, and have divided until they form a conspicuous hemispherical cell-mass projecting into the large lumen of the embryo-sac (*fig. 8*). The number is very great, more than a hundred, or in some older stages a hundred and fifty, thus exceeding, so far as I know, those of any other plants yet investigated. Each cell is clearly defined and is plainly uninucleate. The cytoplasm is very finely granular. The antipodals at this stage recall strongly those of certain Gramineæ.[4] It is very clear that we have to do here, not with a rudimentary structure, but with one which is undoubtedly of physiological importance. The endosperm at this stage is

[4] WESTERMAIER, Zur Embryologie der Phanerogamen, etc. Nova Acta d. kaiserl. Leop. Carol. Akad. der Naturf. 57:1–30. 1890.

scarcely at all developed, and here also, as Westermaier has pointed out in Zea and other grasses, this group of antipodal cells functions as the endosperm in the early stages of development after fertilization.

The nuclei in the antipodal cells of Sparganium never show any indications of fragmentation, such as has been described for the large antipodal cells of some Ranunculaceæ,[5] but they remain sharply defined, and the large nucleolus persists unchanged so far as has yet been observed.

Later there is the usual formation of cell-walls between the endosperm-nuclei, and the development of the endosperm proceeds centripetally. In the oldest stages examined (*fig. 9*), the large mass of antipodals was still evident, although there were some indications of breaking down in these cells. Whether traces of them can be detected in the ripe seed remains to be seen.

The development of the embryo of Sparganium has been already followed in *S. ramosum* Huds. by Hegelmaier,[6] but he gives no account of the embryo-sac. My own studies on the embryo are still too incomplete to make a comparison with the results of Hegelmaier possible. The embryo has the suspensor only slightly developed and there are a number of suggestive resemblances to the embryo of the grasses, which, in connection with the strong similarity in the embryo-sac, point to a possible connection between the Sparganiaceæ and Gramineæ.

As already indicated, it is among the Gramineæ that we find the type of embryo-sac which resembles most that of Sparganium. Hofmeister[7] was the first to call attention to the conspicuous antipodal cells in certain Gramineæ, and showed that the number might exceed the three usually found in angiosperms. Among the Triticeæ he found the number to be from six to twelve.

[5] MOTTIER, Contributions to the embryology of Ranunculaceæ. BOT. GAZ. 20:241, 248, 276, 304. 1895.

[6] Zur Entwickelungsgeschichte monocotyledoner Keime. Bot. Zeit. 32:631. 1874.

[7] Embryobildung der Phanerogamen, II. Monocotyledonen. Abhandl. d. königl. sächs. Gesell. d. Wiss. 7:—. 1861.

The later work of Westermaier,[8] Fischer[9] and Koernicke,[10] has confirmed the results of Hofmeister's investigations and added a good deal to them. Of these investigators, Koernicke alone gives definite information as to the number of antipodal cells which may occur. According to his statements, the number may rise to thirty-six or even more, the largest number, so far as I know, yet recorded, but, as we have seen, very much smaller than the normal number in the older embryo-sac of *Sparganium simplex*. The most noticeable difference, aside from the number of the antipodal cells, is the very inconspicuous character of these before fertilization in Sparganium when compared with the antipodal cells of the Gramineæ.

Lysichiton.

Lysichiton Kamtschatcense (L.) Schott is a monotypic aroid of northeastern Asia and Pacific North America where it extends from Alaska to northern California. In general appearance it is much like the eastern skunk-cabbage, and it is locally known by the same name. Its inflorescence, however, is quite different and recalls that of some species of Anthurium; indeed, the whole plant resembles some of the larger species of this tropical American genus.

I am indebted to Professor Hill of the University of Washington for material of the young flowers and fruit, which he kindly sent me from Seattle. Material of the older stages was collected by me in the neighborhood of Sitka, where the plant grows in great abundance.

The Araceæ have been somewhat neglected in the numerous embryological studies that have been made upon the angiosperms, and, aside from Hofmeister's researches, there is very little work upon the development of the embryo-sac. Fischer[11]

[8] Westermaier, *loc. cit.*

[9] Fischer, A., Zur Kenntniss d. Embryosacentwickelung einiger Angiospermen. Jenaische Zeitschrift 14:—. 1880.

[10] Koernicke, Verhandl. d. naturhistor. Vereins d. Preuss. Rheinlande, etc. 53: 149. 1896.

[11] Fischer, *loc. cit.*

gives some account of the development of the ovule and embryo-sac in Arum, and Mottier has investigated the same points in Arisæma,[12] but beyond these I have not been able to find any references to the embryo-sac in this very characteristic group of plants. As the work of Hofmeister had already shown that the Araceæ exhibit certain marked peculiarities of the embryo-sac, it is remarkable that these plants have been overlooked in the more recent studies of the embryo-sac. The Araceæ give many indications of being a primitive and generalized order, and no group of angiosperms is likely to furnish more important data as to the origin of the structures of the embryo-sac.

In addition to the study of Lysichiton, the more important results of which are here given, more or less extensive studies have been made upon a number of tropical aroids, but these are not yet completed. The forms examined included species of Anthurium, Philodendron, and Dieffenbachia, collected in Jamaica during the summer of 1897.

The ovule of Lysichiton is very large (*fig. 10*) and characterized by the massive basal part, which is extraordinarily large when compared with the nucellus. In the younger ovule the outer integument is only slightly developed, but later it grows out beyond the inner one (*fig. 15*). The embryo-sac is deep-seated. So far as its early development has been followed, it does not differ from that of the typical angiosperms, and there are found the usual structures of the mature embryo-sac, *i. e.*, the egg-apparatus, polar nuclei, and three antipodal cells. While the embryo-sac itself is decidedly smaller than in Sparganium, the egg-apparatus and antipodals are much larger.

At first the nuclei of the cells of the egg-apparatus and the antipodal cells are much alike (*fig. 11*), but at the time of fertilization (*figs. 12, 14*) the antipodal nuclei have increased remarkably in size, while there has been little change in those of the egg-apparatus, although both the egg-cell and synergidæ

[12] MOTTIER, On the development of the embryo-sac in Arisæma triphyllum. BOT. GAZ. 17: 258. 1892.

have increased in volume. As in Sparganium, the latter are much more granular than the egg-cell, but there is less difference in size than is the case in Sparganium.

In the specimen which is shown in *fig. 14*, which was probably ready for fertilization, the two polar nuclei were still separate, but whether they fuse before fertilization is effected must remain for the present undecided.

The subsequent history of the embryo-sac in Lysichiton differs strikingly from that of Sparganium. There is a formation of free nuclei resulting from the division of the primary endo sperm-nucleus, but this is early followed by the formation of division-walls which extend entirely across the lumen of the embryo-sac, and this soon becomes completely filled with the large-celled endospermal tissue (*figs. 15, 16*). This filling of the embryo-sac with a continuous tissue seems to be characteristic of most of the aroids hitherto examined, and recalls the structure of the prothallial tissue in Selaginella or Isoëtes. The same type of endosperm has also been observed in various dicotyledons.

In Lysichiton, as in Sparganium, there is a marked growth of the antipodal cells subsequent to fertilization. They enlarge rapidly and divide, but the number is never large (the specimen figured showed eight). The cytoplasm of the antipodal cells is finely granular, and they are doubtless of physiological importance. Sometimes, as in the case figured (*fig. 17*), the antipodals are clearly separated from the basal endosperm cells which are much smaller; but this is not always the case.

The nuclei of the older antipodal cells become very large, and the conspicuous nucleolus of the younger nucleus becomes much less evident. The actual division of these nuclei was not seen, but the appearance of the chromatin in the cells shown in *figs. 12* and *13* indicates that they are preparing for division. The nuclei finally reach an extraordinarily large size, and far exceed those of the endosperm-cells. They are probably finally disorganized, but their further history was not followed. Whether fragmentation occurs, as in the large multinucleate antipodal cells of some

Ranunculaceæ[13] is doubtful, but there is little question that the early divisions are karyokinetic, and each division is followed by the formation of a division-wall.

The upper endosperm cells are usually decidedly larger than those which are in contact with the antipodal cells, but otherwise there is no marked difference between them.

As in the other aroids that have been studied, the upper part of the nucellus persists as a sort of cap covering the apex of the embryo-sac; the lateral tissue of the nucellus, however, is completely disorganized, so that the embryo-sac comes in close contact with the inner integument (*figs. 15, 18*).

So far as my observations go, the species of Philodendron, Anthurium, and Dieffenbachia examined agree closely with Lysichiton in the development of the embryo-sac, but as yet the presence of such conspicuous antipodal cells has not been observed. In many cases noted (see *fig. 18*) the embryo-sac, at a very early period, becomes completely filled with large-celled parenchyma, in which it is impossible to recognize any antipodal cells, nor in many cases can any certain traces of an embryo be detected. It is hoped that a more thorough study of these puzzling forms, which I expect to make soon, will explain the meaning of these appearances.

Except in Pistia, which has been carefully studied by Hegelmaier,[14] I have been unable to find any complete account of the development of the embryo among the Araceæ. Hofmeister gives figures of a few stages of a number of genera, but these are too incomplete to be of much value. A preliminary examination of the embryo of Lysichiton indicates that it is of much the same type as that of Pistia. Probably no suspensor is developed, and the young embryo is very soon completely imbedded in the endosperm, recalling the appearance of the embryo in Isoëtes (*fig. 16*).

The embryo finally completely fills the ripe seed, as it does in many other genera, *e.g.*, Anthurium, but in some of the aroids,

[13] MOTTIER, *loc. cit.*

[14] HEGELMAIER, *loc. cit.*

e.g., Philodendron, the embryo is smaller and surrounded by abundant endosperm.

SUMMARY AND CONCLUSIONS.

In both Sparganium and Lysichiton there is a normal multiplication of the original three antipodal cells, although the type of these cells is quite different in the two genera. In Sparganium, at least in *S. simplex*, the number of antipodal cells becomes very great, in extreme cases 150 or more, thus far exceeding that of any other known plant. The nearest approach to this is found in certain Gramineæ, where the form of the antipodal cells may be also much like those of Sparganium.

This extraordinary development of the antipodal cells in Sparganium, however, is secondary, and dependent apparently upon the fertilization of the egg-cell. In the unfertilized embryo-sac, the antipodals are unusually small and inconspicuous, and of the usual number. There is no evidence that the nuclei of the antipodal cells divide otherwise than by karyokinesis.

In Lysichiton the antipodal cells probably never exceed ten, but they become extremely large and the nuclei reach enormous proportions. Here, also, no direct division of the nucleus could be detected, but indications of degeneration were observed in the older nuclei, and it is possible that there may be, finally, a fragmentation like that found in the multinucleate antipodal cells of other plants. The character of the antipodal cells in Lysichiton is quite different from those of Sparganium, and, indeed, from those of any other described angiosperms. They resemble most, perhaps, such Compositæ as Senecio and Aster, in which there have been described numerous antipodal cells and a continuous endosperm. In these composites there may be also a multiplication of the nuclei of the individual antipodal cells.

It is evident that we can no longer regard the antipodal cells as merely vestiges of the primitive prothallial tissue. It is true that they probably represent this tissue, but that they may still be of importance physiologically is amply shown both by their great development in the types under consideration, and by researches bearing upon their relation to the nutrition of the structures in the embryo-sac.[15]

Whether the striking resemblances in structure between the antipodal cells of Sparganium and those of certain grasses indicate any direct relationship between the Sparganiaceæ and Gramineæ, remains to be seen; but in connection with the undoubted similarities in the structure of the embryo, it is by no means improbable that these two supposedly isolated orders of the monocotyledons may really prove to be related.

That two such widely separated orders as Araceæ and Compositæ should show analogies in those structures which may very properly be assumed to be of importance as indicating relationships, seems very surprising. It must be remembered, however, that the Compositæ seem to have retained a very primitive type of ovule, *i. e.*, the solitary terminal ovule such as appears to be the most primitive type known in the angiosperms. This same type also occurs in many aroids, and is found again in the simpler Centrospermæ among the dicotyledons, *e. g.*, Polygonaceæ. In the Piperineæ, which show a number of curious analogies with the Araceæ, the same solitary axial ovule occurs. It is not impossible that a further study of Piperineæ may show that in them we have a point of contact between monocotyledons and dicotyledons. If it should be shown that the single ovule of the Compositæ is a primitive rather than a secondary condition, the occurrence of what seems to be a primitive type of embryo-sac in these most specialized of all angiosperms would be comprehensible.

Of course with our present very meager knowledge both of the simpler types of dicotyledons and of the aroids, any positive assertions as to the possible relationships of these groups are out of the question. That these groups deserve more careful study than has yet been given them, admits of little question.

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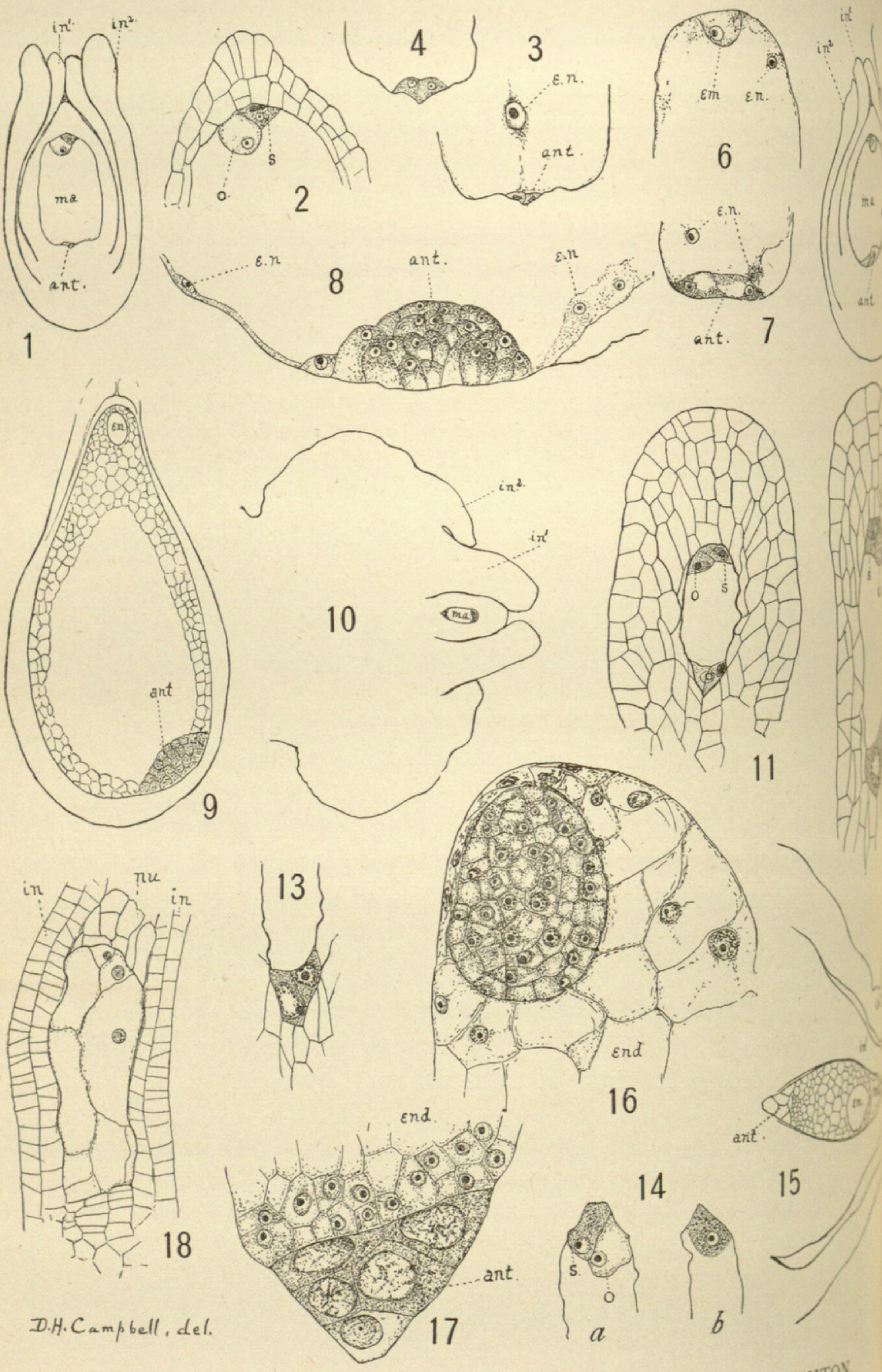
[15] See especially Westermaier's paper already referred to.



CAMPBELL on EMBRYO–SAC of SPARGANIUM and LYSICHITON.

EXPLANATION OF PLATE I.

All of the figures were drawn with a Zeiss camera—*Figs. 1, 5*, Leitz; obj. 3, oc. 3; *figs. 9, 10, 15;* Leitz obj. 13, oc. 1; the other figures, Leitz obj. 7, oc. 1. *Figs. 1–3, 5–9*, refer to *Sparganium simplex*; *fig. 4*, to *S. Greenii; figs. 10–17*, to *Lysichiton Kamtschatcense; fig. 18*, to *Anthurium cordifolium* Kth.

FIG. 1. Section through the mature ovule of *Sparganium simplex; in^1, in^2*, the integuments; *ma*, the embryo-sac.

FIG. 2. Upper part of the nucellus and embryo-sac, showing the egg-apparatus; *o*, the egg; *s*, one of the synergidæ.

FIG. 3. Basal part of the embryo-sac shown in *fig. 2; ant*, antipodal cells; *en*, endosperm nucleus.

FIG. 4. Antipodal cells of *S. Greenii.*

FIG. 5. Longitudinal section of a recently fertilized ovule; the section is cut at right angles to the one in *fig. 1*, so that the funiculus, *fun*, is shown.

FIG. 6. Upper part of embryo-sac, with one-celled embryo, *em*, and one of the secondary endosperm-nuclei, *en*.

FIG. 7. Antipodal region of the same embryo-sac showing two of the enlarged antipodals and two endosperm-nuclei, *en*.

FIG. 8. Antipodal region of an older embryo-sac, showing the large mass of antipodal cells, and the thin parietal layer of protoplasm with the included endosperm nuclei.

FIG. 9. Longitudinal section of an older ovule, showing the embryo-sac lined with a layer of endosperm, in the upper part of which is embedded the embryo, *em*.

FIG. 10. Longitudinal section of a young ovule of Lysichiton.

FIG. 11. The nucellus of the same more highly magnified. The egg, *o*, and one of the synergidæ, *s*, show at the apex, and two antipodals at the base of the embryo-sac.

FIG. 12. Longitudinal section of a nearly mature nucellus; one antipodal cell only can be seen.

FIG. 13. The two other antipodal cells from the embryo-sac shown in *fig. 12*.

FIG. 14. Two sections through the apex of a mature embryo-sac of Lysichiton; in *a* the egg and one synergid show, the second synergid shown in *b*.

FIG. 15. Longitudinal section of an older ovule with the embryo, *em*, sur-

1899. "Notes on the Structure of the Embryo-Sac in Sparganium and Lysichiton." *Botanical gazette* 27(3), 153–166. https://doi.org/10.1086/327811.

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