Studies on the Araceae.

The Embryo-sac and Embryo of *Aglaonema* and *Spathicarpa*.

BY

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With Plates XXX, XXXI, and XXXII.
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SEVERAL years ago a series of investigations were undertaken upon the development of the embryo-sac and embryo in the Araceae. Some of the results of these studies have already been published¹, but the investigations were interrupted for a time owing to pressure of other work, and have but recently been resumed. The present paper deals for the most part with two species, *Aglaonema commutatum*, Schott, and *Spathicarpa sagittaeifolia*, Schott.

The earlier literature upon the development of the Araceae has been referred to in the writer's previous papers, and will not be repeated here. A paper by Caldwell upon *Lemna*² was omitted, but except for this the writer has seen no further contributions to the subject.

The materials upon which the present paper is based were collected at the Royal Botanic Gardens at Kew, in August, 1899. Through the kindness of the director, Sir W. Thiselton-Dyer, it was possible to obtain complete series of a number of species from the rich collection of Aroids in the greenhouses at Kew. Some of the material still remains to be investigated.

¹ Annals of Botany, xiv, March, 1900.

² The Life History of *Lemna minor*, Bot. Gazette, xxvii, Jan. 1899.

Nearly all the Araceae that have been studied show certain anomalies in the character of the embryo-sac, and this makes it all the more desirable that further investigations should be made upon this interesting family. Both of the species especially treated in the present paper show more or less marked deviations from the ordinary angiospermous type. It is hoped that further study of other genera of the Araceae may not only add to our knowledge of this family, but may also throw light upon the character and origin of the embryo-sac of the lower Monocotyledons.

Among other forms collected in Jamaica in 1897, was a species of *Aglaonema*, probably *A. commutatum*, cultivated under the name *Dieffenbachia Aglaonema*, which showed some puzzling abnormalities; but the material was too incomplete to make a thorough study of these possible. What was apparently the same thing was found flowering freely at Kew, and a good supply was secured which served for the basis of the work now recorded. Much the same peculiarities of the embryo-sac noted in the Jamaican specimens were found, and a fairly complete study was made of the species.

The other form to which special attention has been given was *Spathicarpa sagittaeifolia*, which was flowering and fruiting very freely in the Kew greenhouses.

Owing to the large amount of mucilaginous matter secreted by the ovules in many Araceae, they are especially difficult to fix properly, and even with the greatest precautions much of the material was very unsatisfactory. On the whole, the best results were obtained by the use of a concentrated alcoholic solution of corrosive sublimate. Aqueous fixing fluids were useless in most cases. Some good results were also obtained by the use of alcohol, to which ten per cent. of acetic acid was added. This was not specially satisfactory for fixing nuclei, but otherwise often gave very good results. Fleming's triple stain was used with success in some cases, but very good preparations were also made by staining with alcoholic Bismarck-brown and aniline-safranin.

Most of the Araceae that have been examined show various

peculiarities, both in the formation of the endosperm, and in the behaviour of the antipodal cells. Some of them, like *Arisaema triphyllum*¹, differ also from the usual angiospermous type, in the character of the archesporium. The archesporium in this species shows in cross-section from two to four or five cells, which, according to Mottier, are the product of the division of a single primary archesporial cell. It seems probable, however, that sometimes, at least, these cannot all be traced back to the division of a single primary archesporial cell, but arise from two or more independent sub-epidermal cells. A similar archesporium has been found by the writer in *Aglaonema commutatum*, and it is quite probable that further research will show the same thing in other Araceae.

Hofmeister² showed that in nearly all the Araceae examined by him the endosperm at an early period fills the embryo-sac with a continuous tissue, or in some cases leaves a greater or smaller portion of the cavity permanently empty³. He however misunderstood the process of cell-division by which the endosperm is formed, and supposed that in all cases there was a 'free cell-formation' preceding the formation of the solid endosperm. It is true that in *Pothos longifolia* he found frequently an early division by a transverse cell-wall; but he infers that the subsequent endosperm formation, which he says is confined to the upper and larger of the two primary cells, is formed by 'free cell-formation,' and not by successive cell-divisions, as is probably the case. A type of endosperm formation somewhat similar to that of the Araceae has been described by Strasburger⁴ for *Ceratophyllum*. In this case, however, the first division-wall in the embryo-sac divides it into equal parts.

Hofmeister called attention to the large size of the antipodal cells in some species of *Arum*, and the writer has found in *Lysichiton* a remarkable development of the antipodal cells

¹ Mottier, Bot. Gazette, 1892.

² Neue Beiträge zur Embryobildung, &c.; Monocotyledonen. Leipzig, 1861.

³ l. c. p. 704.

⁴ Ein Beitrag zur Kenntniss von *Ceratophyllum submersum*, Pringsheim's Jahrb. für wiss. Botanik, xxxvii. 504, 1902.

subsequent to fertilization, and much the same thing occurs in *Spathicarpa*, and probably in other Araceae as well. On the other hand, in *Aglaonema commutatum*, it was often impossible to demonstrate certainly the presence of any antipodal cells, although in the apparently closely related species, *A. pictum*, they seem to be always present.

The development of the embryo in the two species under consideration does not show anything specially noteworthy. In *Spathicarpa* the embryo remains small, and there is a largely developed endosperm in the ripe seed ; in *Aglaonema* the mature embryo nearly fills the embryo-sac.

The very considerable variation shown in the types of Araceae already studied make it highly desirable that as many types as possible should be investigated in order to determine the affinities of the family. It is to be hoped that the characteristic genera of the eastern United States may be examined by some of the botanists who are interested in the morphology of the embryo-sac, and have access to material of our native Araceae.

AGLAONEMA.

The genus *Aglaonema*¹ comprises about ten species of the East Indies and Malayan region. Several species, including *A. commutatum*, are in cultivation. The latter species was flowering freely at Kew in August, 1899, and apparently the same plant was collected at the Hope Gardens, near Kingston, Jamaica, in the summer of 1897. A second species, *A. pictum*, was also flowering at Kew, and material of this species was collected.

The flowers are unisexual, the pistillate flowers being borne at the base of the thick spadix, the crowded staminate ones occupying the upper portion. No perianth is developed, but there is a conspicuous white spathe, partially enwrapping the base of the spadix.

The pistillate flower consists of a single carpel, with a

¹ Engler and Prantl, Die natürlichen Pflanzenfamilien, 11. Th., 3. Abth., p. 135.

large solitary anatropous basal ovule. The nearly globular ovary is crowned with a large discoid sessile stigma, slightly depressed in the centre, where it joins the very short canal leading to the ovarian cavity.

While *A. commutatum* and *A. pictum* agree closely in their structure, the former, owing to its larger nuclei, as well as from the peculiarities of the embryo-sac, was especially studied, and, unless otherwise stated, the account here given refers to this species.

The ovule from the first is very massive, and while the integuments are developed at an early period, they remain short, the chalazal region of the ovule being very large—a not uncommon feature in other Araceae as well. The short funiculus is also very thick.

The nucellus is relatively small. In the youngest stage observed (Pl. XXX, Fig. 1) the young embryo-sac was already evident, an elongated cell with a large nucleus. Whether in this case the embryo-sac arose directly from a hypodermal cell, or whether it was the product of the division of a primary archesporial cell, could not be positively ascertained.

The lateral tissue of the young nucellus consists of two or three layers of cells, while at the summit the cells are somewhat larger, and persist as a cap of tissue after the lateral tissue of the nucellus is destroyed. This occurs at an early stage, so that the embryo-sac soon comes into contact with the inner integument.

In a number of cases observed, and this evidently is not unusual, the archesporium consisted of two or three large cells, all of which were apparently potential embryo-sacs. These ovules (Figs. 5, 7) much resemble those of *Arisaema triphyllum*.

As the embryo-sac enlarges the lateral tissue of the nucellus is soon crowded upon, and finally becomes quite obliterated. The apex of the nucellus, as in many other similar cases, persists as a conical cap above the apex of the embryo-sac.

There is so much variation in the behaviour of the embryo-sac in its earlier stages that it is difficult to say what may be

considered the normal development. No tapetal cells were observed in any case, and the single archesporial cell, or each of the two or three cells where more than one embryo-sac mother-cell is present, develops at once into the embryo-sac. Where two or three young embryo-sacs develop, they may be separated by an obliquely transverse wall (Fig. 7), or more commonly by oblique or longitudinal walls (Fig. 5).

Where a single embryo-sac only is present, it enlarges rapidly, and the nucleus divides as usual, the two daughter-nuclei sometimes at least occupying approximately the micropylar and antipodal ends of the young sac (Fig. 4). With the crowding upon the nucellar cells, their nuclei become extraordinarily flattened, assuming the form of thin discs, in which the nuclear network is extremely conspicuous (Fig. 4, *c*). After the second nuclear division in the embryo-sac, the nuclei may be in pairs at the end of the sac, but this is not always the case. In the specimens shown in Fig. 6 the four nuclei were close together at the apex of the sac, and from other cases examined it is clear that the polarity, so marked in the embryo-sac of most Angiosperms, is in this case very slight or quite absent. Even where the nuclei are in two groups they are more often placed laterally than at the extremities of the sac. This is especially true of the group from which the egg-cell develops. So far as could be determined from the examination of a large number of ovules, the characteristic structures of the typical angiospermous embryo-sac, the egg-apparatus, polar nuclei, and antipodals are never clearly differentiated in *Aglaonema commutatum*.

While the number of nuclei is probably eight in most cases, there are frequently deviations from this number. In the specimen shown in Fig. 8, *c, d, e*, there were twelve nuclei, in three groups of four; Fig. 18 shows a sac with ten nuclei, six in one group and four in the other; in Fig. 9 is shown a sac in which the nuclei were only four, although something like an egg-apparatus was evident at the micropylar end of the sac.

Where the archesporium consists of more than one cell,

each is a potential embryo-sac, and its nucleus may undergo the first divisions; but in no case observed was more than one complete embryo-sac seen. The secondary embryo-sac, where two are present, may persist, however, until the definitive embryo-sac has reached its full development; indeed it is quite impossible sometimes to be certain whether the structures present at the time of fertilization are all the products of a single embryo-sac, or of two (see Fig. 11). Where two or three embryo-sac mother-cells are present, they are often quite similar, and as the nucleus may divide once or twice in all of them, it is impossible to determine which is destined to become the definitive embryo-sac. In Fig. 5 is shown an ovule where there are two entirely similar young embryo-sacs, each of which contains four nuclei. In another case observed (Fig. 7) there were three young embryo-sacs separated by oblique walls. In the upper one (*a*) there was a single large nucleus; in the second (*b*) there were eight nuclei, in two groups of four, and the members of each group partially fused, in a manner entirely similar to the ordinary fusion of the polar nuclei. The lower sac (*c*) was apparently the definitive embryo-sac. There were eight (possibly nine) nuclei in this. Four of the nuclei were enclosed by delicate membranes, and formed a group of cells suggesting an egg-apparatus, or possibly a group of antipodal cells. Near these was a nearly hemispherical cell (*d*) whose nucleus was preparing to divide. This was near the upper end of the sac, but at one side. Near the base of the sac was a group of large nuclei, partially fused together, and presumably giving rise to the endosperm-nucleus. Three nuclei were very plainly seen, and there was possibly a fourth one, but this could not be certainly determined. Whether the two groups of nuclei in the second embryo-sac (*b*) were destined to take part in the endosperm formation, could not, of course, be determined.

In the embryo-sac shown in Fig. 10 there were eight nuclei. Four of them were at one side of the sac near the apex, and there was some indication of the differentiation of

an egg-cell. The other four were at the antipodal end of the sac, but there were no clearly indicated antipodal cells, nor was there, at this stage, any indication of polar nuclei. A second embryo-sac, containing two nuclei, accompanied this one.

Fig. 23, which shows an embryo-sac taken from the Jamaican specimens, differs from any cases found in the material collected at Kew. At the micropylar end of the sac was a well-marked egg-apparatus, consisting of two clearly defined synergidae and an egg-cell. At the chalazal end were two large nuclei surrounded by a mass of granular cytoplasm, and evidently in the early prophase of division. It would certainly seem that in this case no polar nuclei could be developed, and from older specimens from the same plant (Fig. 24) it looks very much as if the basal nuclei assumed at once the rôle of endosperm nuclei, proper antipodal cells being quite suppressed.

The specimen figured as Fig. 18 showed ten nuclei. Four of these were at the base of the sac, where there was developed a group of four cells, one of which projected into the cavity of the sac, and may have been the egg-cell, but it is possible that this group of four cells may have been antipodal cells. Nearly opposite these were six free nuclei, but no further differentiation could be discerned, and what the further history of the sac would have been could only be conjectured.

In the embryo-sac shown in Fig. 12 there were twelve nuclei. In this case the pollen-tube could be seen, but it was not certain which were the generative nuclei. Above the sac was a second one, in which were two conspicuous nuclei, which may possibly have been the generative nuclei derived from the pollen-tube, but they were much more probably the nuclei of the second embryo-sac. The apex of the lower embryo-sac contained no nuclei. At the lower end were four nuclei (*b*) with delicate membranes between them, and probably to be considered as antipodal cells. Near them was a second group of four cells, which may have represented the egg-apparatus, and on the opposite side of the sac were four

free nuclei, apparently in process of fusion (*c*), presumably to form the endosperm-nucleus.

The specimen drawn in Fig. 13 showed a group of four cells at the apex of the sac. Of these, two lying side by side (*o*, *o'*) were hemispherical, and probably one of them was the egg. In contact with this group of cells was the enlarged end of the pollen-tube, containing a nucleus, which was probably one of the generative nuclei. The second generative nucleus was not clearly visible. Nothing resembling antipodal cells could be seen, but four free nuclei (Fig. 14) were seen in the cavity of the embryo-sac. Two of these were larger, and in close contact. The other two showed some indications of disorganization.

Fig. 15 shows a very peculiar case, which was probably abnormal. There were apparently three embryo-sacs, two smaller ones above and the larger definitive one below. The two upper ones showed signs of degeneration. In the lower one, the chalazal end of the sac was occupied by two very large irregular nuclei (Fig. 16) having every appearance of being composed of several nuclei fused into one. Two other nuclei were present, one of which (Fig. 17) also looked as if it were compound.

In Fig. 11 is shown a puzzling case. Separated from a large cell below, were two distinct cells which may have been derived secondarily from a division of the primary embryo-sac, or may perhaps represent two other embryo-sacs. There was no sign of degeneration in these, and with the large lower cell they seemed to form one structure. Traces of the pollen-tube could be seen, and in one of the cells two nuclei could be seen which were possibly the generative nuclei. Besides these nuclei, two others were present, and in the second of the upper cells there were three nuclei. In the lower cell were two large nuclei in close apposition.

It is very evident that in *Aglaonema commutatum* we have to do with an extraordinarily variable plant. The not infrequent increase in the number of the embryo-sac nuclei, and the imperfect differentiation of the usual structures of the

embryo-sac, are worthy of note, as is also the absence of marked polarity in the arrangement of the nuclei. The frequent occurrence of multiple nuclear fusions is also interesting, as in this respect this species furnishes a condition intermediate between that found in *Peperomia* and that occurring in the typical Angiosperms. A similar condition has been found to exist also in the peculiar genus *Gunnera*¹.

Where the egg-cell was evident it was usually hemispherical, and was either at the apex of the sac or, more often, laterally placed. While the pollen-tube was seen in a few cases, the fertilization was not satisfactorily made out, and it is possible that some of the apparently abnormal appearances encountered may have been due to lack of fertilization.

The development of the embryo is slow at first, and it remains very small until the endosperm is well advanced in its development. It is not rare to find the embryo-sac filled with a solid mass of endosperm, without any certain evidence of an embryo being present at all.

THE ENDOSPERM.

It is not probable that the formation of the endosperm is entirely uniform in *Aglaonema commutatum*. To judge from the frequency with which multiple fusions of the nuclei of the embryo-sac are encountered, it is likely that in such cases the primary endosperm-nucleus results from such fusions, although it also probably may arise from the fusion of two nuclei, as in most Angiosperms. The definitive endosperm nucleus was not seen, nor was it possible to find the first division: but to judge from a comparison with *Spathicarpa* and *Anthurium*, in which the young endosperm presents much the same appearance, it is probable that the first division is accompanied by a wall which cuts off a relatively small cell from the base of the embryo-sac. This is followed by a similar division in the cavity of the embryo-sac, and

¹ Schnegg, Beiträge zur Kenntniss der Gattung *Gunnera*, Flora, Bd. 90, pp. 161-208 (1902).

a second cell is cut off from its base, in contact with the first one. In this way the formation of a solid endosperm proceeds from the base upwards (Fig. 21) until the whole cavity is filled. These cells later undergo further divisions, but in all cases, apparently, nuclear divisions are accompanied by cell-formation.

Not infrequently a group of cells, differing somewhat in appearance from the endosperm-cells, can be seen at the base of the embryo-sac (Figs. 27, 32). These may be possibly antipodal cells, but this point was not satisfactorily proven, and it is not impossible that in some cases, at least, they are merely somewhat modified endosperm cells.

The embryo-sac shown in Fig. 23 was found in some of the Jamaican material referred to this species, but perhaps not correctly determined. In this case, as we have already stated, besides the egg-apparatus, there were but two nuclei, situated at the base of the embryo-sac, and evidently in the early prophases of division. A somewhat older embryo-sac (Fig. 24), evidently of the same type, was found, and from a comparison with these, it appears that the endosperm formation results directly from the further division of the two basal nuclei found in the younger sac. This may be a further development of the type shown at Fig. 9, where the three apical nuclei were already arranged like an egg-apparatus, while but a single nucleus occupied the base of the sac. The complete absence of antipodal cells and polar nuclei in these instances, and the development of endosperm without the preliminary nuclear fusion, is certainly noteworthy.

The embryo-sac in *Aglaonema* becomes strongly bent as it grows. The peculiar mass of cells referred to, differing in appearance from the endosperm-cells (Figs. 27, 32), while in some cases to be interpreted as a mass of antipodal cells, may possibly be an embryo in some instances, as unmistakable young embryos have been found at the chalazal end of the sac; and in some of these embryo-sacs of large size, and already filled with endosperm, no trace of an embryo can be detected at the micropylar end.

The embryo-sac in the ripe seed occupies relatively a small part of its bulk. The large development of the integuments and chalazal part of the ovule suggests the perisperm formation of the Piperaceae or Cannaceae.

THE EMBRYO.

The embryo may occupy the usual position at the micropylar end of the sac, but more commonly it is at some distance from the apex of the sac, corresponding to the lateral or even basal position of the egg-cell.

The first divisions (Fig. 24) are probably always transverse, but no well-defined suspensor is developed, this doubtless being associated with the early filling up of the sac with endosperm, which completely invests the young embryo. The cell next the wall of the embryo-sac may be regarded as a suspensor cell, but it does not become enlarged, nor give any other evidence of being functionally important.

The subsequent divisions of the embryo do not show an absolute regularity. The strikingly pointed form of the young embryo shown in Fig. 26 recalls the embryos of some Grasses; but this does not appear to be by any means always characteristic of the young embryo of *Aglaonema*.

As the embryo grows it assumes an elongated form, but for a long time there is no differentiation of the external parts, nor are the tissues at all clearly defined. The enlarging embryo encroaches rapidly upon the endosperm, and fills almost completely the upper part of the embryo-sac. The greater part of the embryo is composed of the cotyledon, which becomes somewhat club-shaped and expanded at the end, which crowds into the enlarged chalazal part of the embryo-sac, destroying the endosperm as it grows, and leaving but a small part of it intact (Fig. 33). The tissues of the embryo are almost perfectly uniform, and the boundaries of the different organs very vaguely defined. The hypocotyl is very short, and although a median section of the root-end of the embryo (Fig. 31) shows some slight differentiation of the primary tissues, this is very imperfect. In the older embryo

(Fig. 34 *a*), a single layer of root-cap cells could be seen, but the tissues of the root-apex show very little evidence of a definite arrangement, and the same is true of the stem-apex (*b*), whose exact position it is impossible to determine.

AGLAONEMA PICTUM.

A brief examination was made of *A. pictum*, which closely resembles in general appearance *A. commutatum*, but which shows notable differences in the development of the ovule. The cells of the ovule are decidedly smaller, with correspondingly small nuclei, so that the two species are readily distinguishable in this way. More important, however, is the marked difference in the development of the embryo-sac. So far as one can judge from an examination of a considerable number of mature embryo-sacs of *A. pictum*, this species shows none of the variability so characteristic of *A. commutatum*. In all the specimens examined, the embryo-sac was not essentially different from other typical Angiosperms. The hemispherical egg-cell was accompanied by two large and conspicuous synergidae. Small but perfectly characteristic antipodal cells were at the base of the sac, and a single large endosperm-nucleus, evidently the product of the fusion of two polar nuclei, was conspicuous. Numerous starch granules were present in the sac, especially around the endosperm-nucleus—a not unusual feature of the embryo-sac in some other Araceae, e.g. *Dieffenbachia*. Except for the slightly lateral position of the egg-apparatus, *A. pictum* conforms entirely to the ordinary angiospermous type, and it is very remarkable that the apparently closely related *A. commutatum* should show such an extraordinary difference in the development of the embryo-sac.

The ripe pollen-spores of *A. pictum* contain two generative nuclei, in which respect they differ from those of *Dieffenbachia seguine*, where there is but a single generative nucleus. *Symplocarpus*¹ also shows but a single generative nucleus in the ripe spore.

¹ Duggar, Bot. Gazette, xxix, Feb. 1900.

SPATHICARPA SAGITTAEFOLIA.

The genus *Spathicarpa*¹ includes four very characteristic South American Aroids. The flowers, which are of the simplest possible structure, are borne directly upon the upper surface of the green leaf-like spathe, no spadix being developed. The flowers are arranged in rows, staminate and pistillate flowers being intermingled. The staminate flower consists of a peltate synangium borne upon a stalk, and closely resembles the peltate sporophyll of *Equisetum*. The pistillate flower (Fig. 35) consists of a single peg-shaped carpel, terminating in a small stigma. No perianth is present, but between the flowers are numerous small nearly sessile staminodia.

The great simplicity of the flowers suggested that the embryo-sac might possibly show correspondingly primitive characters, but this was not found to be the case, although there were some interesting peculiarities which will be considered presently.

The material used, like that of *Aglaonema*, was collected at Kew, and all belonged to one species, *S. sagittaeifolia*. A brief reference to the structure of the embryo-sac in this species has already been published², but a mistake was made in the interpretation of certain structures, which has since been corrected.

The peg-shaped carpel of the exceedingly simple flower is tipped by a small stigma composed of the usual papillate cells. The short style merges gradually into the ovary, which is completely filled by the single basal orthotropous ovule (Fig. 35). Both integuments are well developed, and, as in *Aglaonema*, the young embryo-sac soon destroys the lateral tissue of the nucellus, and thus comes into direct contact with the inner integument.

The earliest stages of the embryo-sac were not seen, but from the youngest ones which could be found it is evident

¹ Engler and Prantl, l. c., p. 145.

² Campbell, American Naturalist, Oct. 1902.

that there is no marked departure from the usual course of development. Whether the embryo-sac mother-cell arises at once from the primary archesporial cell, or is formed after division of the latter, could not be determined.

The apical part of the nucellus persists as in *Aglaonema*, but is perhaps a little larger, relatively. In the youngest case met with (Fig. 36) the primary nucleus of the embryo-sac had already divided, and the daughter-nuclei, which were placed at opposite ends of the sac, were dividing, the division of the two taking place simultaneously, and the embryo-sac at this stage presented the usual appearance.

No attempt was made to follow in detail the development of the sac up to the time of fertilization, as it was evident that it was in no way peculiar. The three cells of the egg-apparatus are nearly similar, and taper into the narrowed micropylar end of the sac. The polar nuclei are just above the three antipodal cells (Fig. 39), and their fusion into the endosperm nucleus is completed some time before fertilization takes place. In most cases observed, the fusion was complete. The antipodal cells are surrounded by distinct membranes, and while not noticeably conspicuous, are readily demonstrable.

Owing to the small size of the nuclei the plant does not offer special facilities for studying the phenomena of fertilization, and no attempt was made to follow these in detail, although in several cases the sexual nuclei were observed in process of fusion. The process is evidently slow, and before it is complete the egg has increased perceptibly in size, and the pollen-nucleus, which is smaller than the egg-nucleus, becomes much flattened against the latter, with which it finally merges completely (Figs. 41, 44). The fusion-nucleus is not noticeably rich in chromatin, but has a conspicuous nucleolus. In the cytoplasm of the egg there are formed numerous small starch-grains which become larger before the first division occurs in the young embryo. These starch grains disappear during the early cell-divisions in the embryo, and are probably used in the formation of the cell-walls.

In the specimen figured in Fig. 37 one of the synergidae was conspicuous, while the second one was smaller, with a smaller nucleus; whether this inequality is frequent in *Spathicarpa* was not determined.

THE ENDOSPERM.

The endosperm-nucleus increases a good deal in size before its first division. It lies just above the antipodal cells, and is imbedded in a mass of granular cytoplasm, which exhibits a more or less reticulate appearance in sections, probably indicating a vacuolate structure in the living state. The endosperm-nucleus has a conspicuous nucleolus, and is much richer in chromatin than the egg-nucleus. The first division of the nucleus was not seen, but from the appearance of the very young endosperm there is little question that the first division results in the cutting off of a basal endosperm-cell from the embryo-sac. The youngest stage observed (Fig. 40) consisted of three basal endosperm-cells, with exceedingly delicate membranes, and a fourth nucleus lying free in the base of the undivided portion of the embryo-sac. From a study of older stages, it is clear that the development of the endosperm proceeds from the base toward the apex of the sac, which soon becomes entirely filled with the cellular endosperm. The large, thin-walled primary endosperm-cells divide further, so that the cells of the endosperm become smaller and their membranes thicker.

THE ANTIPODAL CELLS.

The three small antipodal cells present at the time of fertilization are stimulated into active growth and show an extraordinary development. Not infrequently, in somewhat later stages, four or occasionally more antipodal cells are present, but it is probable that the increased number is due to a division of one or more of the original antipodal cells, subsequent to fertilization. The small compressed antipodal cells of the embryo-sac at the time of fertilization elongate

rapidly to many times their original dimensions, and show every indication of extremely active growth. The cytoplasm is abundant, and the small nuclei enlarge rapidly, becoming extremely conspicuous. At this period (Fig. 42) the enlarged antipodal cells so closely resemble the young endosperm that they were at first mistaken for them, and this led to the erroneous statement made in a former article.

The walls of the antipodals become thicker, and they ultimately reach gigantic dimensions, being easily seen in sections by the naked eye (Fig. 56). This enlargement is accompanied by a corresponding growth of their nuclei, which become many times larger than those of the endosperm-cells. At first they show a perfectly normal structure, but later there are evidences of disintegration. The nucleoli become immensely enlarged (Fig. 59), and finally very irregular in form, and the reticulate structure of the active nucleus becomes more or less completely broken down, and shows many evidences of disintegration.

From their close resemblance to the active young endosperm-cells, there can be no doubt that the antipodals function as endosperm during the early development of the fertilized embryo-sac.

The great enlargement of the antipodal cells is not peculiar to *Spathicarpa* among the Aroids. Hofmeister¹ figures enlarged antipodal cells in *Arum orientale*, and the writer has noted the same phenomenon, accompanied by increase in their number, in *Lysichiton*. In the latter there is also the great enlargement and subsequent degeneration of the nuclei of the antipodal cells. It is highly probable that a similar condition will be found in other genera.

THE EMBRYO.

The fertilized egg increases slowly in size, but there is no division of the embryo until the embryo-sac is nearly or quite filled with endosperm, so that almost from the first the embryo is completely surrounded by the endosperm-tissue,

¹ l. c., Pl. VII, fig. 4.

and, as might be anticipated, the suspensor is very rudimentary.

The first division is transverse and cuts off a small pointed basal suspensor-cell, which, however, undergoes very little further growth. The next divisions are probably not always exactly the same. Usually the second wall is vertical (Fig. 45) and divides the embryonal cell into two nearly equal parts. Sometimes before this vertical wall is formed an oblique wall may cut off a small cell (Fig. 46) in contact with the suspensor-cell. The latter does not always show the pointed form which usually distinguishes it. Following the first vertical wall in the embryonal cell, there are usually two transverse walls, intersecting it and dividing the embryo into nearly equal quadrants, and these may be divided into octants, although it is not probable that this is always the case. Figs. 49-52 represent nearly median sections of embryos of about the same age, showing the variation in form as well as in the cell-arrangement and the character of the rudimentary suspensor.

For a long time there is no evidence of the development of the external organs, nor is it possible to trace any definite relation between these and the earlier divisions of the embryo. The stem-apex arises in a lateral depression at a point a little below the middle of the embryo, the region below developing the hypocotyl and root, the part above, the cotyledon (Fig. 53). In the origin of the organs of the embryo, *Spathicarpa* does not differ essentially from the commonest type of the Monocotyledons. The tissues of the embryo remain almost perfectly homogeneous, and even in the root, where the arrangement of the tissues is most regular, the limits of the different tissue systems are very imperfectly defined. At the root end, in the most advanced embryo, there may be seen the rudimentary suspensor, and although the tissues are somewhat better defined than in the embryo of *Aglaonema*, still they are rather vague. The root-cap is not clearly delimited, nor are the meristems below it at all clearly defined. Some evidences of a central strand can be made out, above which

is a layer of meristem, which probably serves as the initial for all the outer tissues.

The epidermal cells of the stem-apex are narrower than the ordinary epidermal cells (Fig. 55), but otherwise the stem-apex is indistinguishable, except from its position. The cotyledon constitutes the greater part of the embryo, and its base partially encloses the inconspicuous stem-apex, as is so often the case among Monocotyledons.

None of the permanent tissue elements were recognizable in the oldest embryos which were available, and only the slightest traces of the young vascular bundles could be detected. The embryo in the ripe seed occupies only a small portion of the large embryo-sac, which is filled with the endosperm-tissue (Fig. 56).

As the seed approaches maturity the endosperm-cells develop numerous small starch-granules. The growing embryo uses some of this, so that the cells immediately surrounding the embryo contain but little starch, while those in the central part of the sac (Fig. 58) contain a great deal. The basal cells of the endosperm become much larger than the upper ones, and their walls are much thickened, so that they show a certain likeness to the enlarged antipodal cells. Like the latter, their nuclei are also enlarged, although not nearly to the same degree, and show some evidences of disintegration.

SUMMARY.

1. In both *Aglaonema* and *Spathicarpa* the pistillate flower consists of a solitary carpel containing a single basal ovule, probably of axial origin.

2. The embryo-sac of *Aglaonema commutatum* shows many deviations from the usual type. These consist first in a varying number of embryo-sacs, ranging from 1 to 3. Where two or three are formed, this may be from a division of a common archesporial cell, but in some cases it looks as if these originated independently from hypodermal cells. All of these embryo-sacs usually undergo the first nuclear divisions, but probably only one ever becomes fully developed. The

second peculiarity is the extraordinary variation in the number of nuclei in the embryo-sac, and in the character of the structures developed in it. The number of nuclei ranges from 4 to 12, and the polarity is usually but slightly indicated. Multiple nuclear-fusions are of common occurrence, and it is often impossible to be certain which of the structures represent the egg-apparatus, and which the antipodal cells.

3. In specimens of an undetermined species (*Dieffenbachia Aglaonema* Hort.), perhaps identical with *A. commutatum*, the endosperm may arise from the direct division of two nuclei (or possibly a single one) at the base of the sac, without any formation of polar nuclei.

4. *A. pictum* does not depart to any marked extent from the usual angiospermous type. The pollen-spore of this species has two generative nuclei.

5. The embryo of *Aglaonema*, although reaching a large size, shows little differentiation of its external parts, and its tissues are almost perfectly homogeneous. In the ripe seed it almost completely fills the embryo-sac. The nucellus is relatively small, but the integuments and base of the ovule are very massive, and comprise the greater part of the seed.

6. In *Spathicarpa* the development of the ovule and embryo-sac are of the usual type. After fertilization the antipodals become very greatly enlarged, and one of them may divide, so that there are often four antipodals present. The nuclei of the antipodal cells become enormously enlarged.

7. The embryo of *Spathicarpa* remains small in the ripe seed. The external organs are indicated, but the tissues remain but slightly developed.

8. The development of the endosperm in both *Aglaonema* and *Spathicarpa* proceeds gradually from the base of the sac until it is completely filled. It is probable that this is the ordinary method of endosperm-formation in the Araceae.

EXPLANATION OF FIGURES IN PLATES XXX, XXXI, AND XXXII.

Illustrating Professor Campbell's Studies on the Araceae.

PLATE XXX.

All figures refer to *Aglaonema commutatum*. Figs. 1, 7, 15, Leitz, oc. 3, obj. 3. Figs. 12, 13, 14, 16, 17, Leitz, oc. 1, oil imm. 1/16; the other figures, Leitz, oc. 1, obj. 7.

Fig. 1. Median longitudinal section of young ovule, showing the young embryo-sac, *m*, and the two integuments *in*₁, *in*₂.

Fig. 2. The nucellus and embryo-sac, more highly magnified.

Fig. 3. Oblique section of the nucellus, showing two young embryo-sacs, apparently derived from the division of a common mother-cell.

Fig. 4 *a*, *b*. Two sections of young embryo-sac with two nuclei. *c*, Nuclei from the lateral cells of the nucellus, more enlarged, showing the very much compressed form.

Fig. 5. Section of a nucellus with two young embryo-sacs; each contains four nuclei.

Fig. 6. Embryo-sac containing four nuclei, all at the micropylar end. (Three only shown in the section.)

Fig. 7. Nearly median section of an ovule with three embryo-sacs.

Fig. 8. The details of the sacs shown in Fig. 7, more highly magnified. *a*, contains a single nucleus; *b*, eight nuclei in two groups, partially fused; *c* *d*, and *e*, the details of sac *c*: *d*, antipodals (?), *e*, egg (?), *c*, fusing endosperm nuclei.

Fig. 9. Sections of embryo-sac with four nuclei, three at the micropylar end, a single one, *b*, at the chalazal end.

Fig. 10. Sections of a sac with eight nuclei, in two groups of four; no definite polar nuclei recognizable.

Fig. 11. Two sections of an ovule with three large cells (embryo-sacs?), but probably abnormal. The largest of the three cells contained but two nuclei (*c*). What looked like a pollen-tube, *p. t.*, occupied the micropyle, above the apex of the nucellus.

Fig. 12 *a*. Upper part of nucellus, showing the pollen-tube, *p. t.* The cell with the two nuclei probably represents a second, imperfect embryo-sac. The lower embryo-sac contained twelve nuclei, in three groups. *b*, the four antipodal nuclei. *c*, four nuclei fusing, presumably to form the endosperm-nucleus.

Fig. 13. Two sections of the apex of a sac, with what seemed to be a very broad pollen-tube, *p. t.*, containing a generative nucleus, *g*. There were four cells at the apex, of which two hemispherical ones, *o*, *o'*, were much alike. One of these is probably the egg-cell.

Fig. 14. Nuclei from the cavity of the embryo-sac shown in Fig. 13. There were four nuclei, two in process of fusion, and two (only one shown in the section) which were apparently disintegrating. No antipodal cells were present.

Fig. 15. Section of a group of three embryo-sacs; the two upper ones degenera-

ting. At the base of the lower one were two very large nuclei, and there were two free nuclei in the cavity of the sac.

Figs. 16, 17. Nuclei from the lower sac shown in Fig. 15 more highly magnified.

PLATE XXXI.

Figs. 18, 22, 28 and 31 refer to *Aglaonema commutatum*, the others to a very similar, but possibly different, species grown at the Hope Gardens, Kingston, Jamaica, under the name *Dieffenbachia Aglaonema*. Figs. 19, 25, 28, 32, Leitz, oc. 3, obj. 3; 33, about 20 diameters; the others Leitz, oc. 1, obj. 7.

Fig. 18. Embryo-sac with ten nuclei, four at the chalazal end (*a*), the other six in a group at one side of the sac (two only shown in the section *b*).

Fig. 19. Section of a fertilized sac, showing the forming endosperm at the base.

Fig. 20. Young embryo, from near the chalazal end of the same embryo-sac.

Fig. 21. Young endosperm of the same sac.

Fig. 22. Group of four [antipodal (?)] cells from the same sac.

Fig. 23. Embryo-sac with egg (*o*) and two synergidae (*c*), at the apex, and two nuclei, preparing to divide, at the antipodal end.

Fig. 24. A similar embryo-sac, after fertilization. At the apex a young embryo, surrounded by large celled endosperm, apparently derived from the division of the basal nuclei shown in Fig. 23.

Fig. 25. An older embryo-sac, with young embryo, *em*.

Fig. 26. Two sections of an older embryo, *sus*, suspensor-cell.

Fig. 27. Base of an older embryo-sac, with mass of tissue different from the endosperm, and possibly representing the embryo, as no other evidence of an embryo can be found. Above this mass was an elongated body (*p. t. ?*), which may possibly have been the pollen-tube.

Fig. 28. Section of embryo-sac, with older embryo, *em*.

Fig. 29. Micropylar (root-)end of an older embryo.

Fig. 30. Apex of the cotyledon of the same embryo.

Fig. 31. Root-end of an embryo, showing slight differentiation of the tissues.

Fig. 32. Embryo-sac, with mass of cells, perhaps an embryo, at the chalazal end. No embryo was present at the apex of the sac.

Fig. 33. Section of older ovule, the embryo filling most of the embryo-sac end, the basal endosperm.

Fig. 34. *a*, root-end; *b*, central portion of the embryo shown in Fig. 33, *st*, the future stem-apex.

PLATE XXXII.

All figures refer to *Spathicarpa sagittae-folia*. Figs. 35, 53, Leitz, obj. 3, oc. 3; Fig. 56, about 8, Fig. 57 about 45 diameters; Figs. 36, 37, 41-44, Leitz, oil imm. 1/16, oc. 1; the others, Leitz, obj. 7, oc. 1.

Fig. 35. Longitudinal section of mature pistillate flower of *Spathicarpa sagittae-folia*; *m*, embryo-sac.

Fig. 36. Young embryo-sac; the second nuclear division is taking place; the chalazal nucleus was also dividing.

Fig. 37. *a*, egg-apparatus; *b*, endosperm-nucleus from a mature embryo-sac.

Fig. 38. Antipodal cells and endosperm-nucleus, from an embryo-sac of about the same age as Fig. 37.

Fig. 39. Antipodal cells and polar nuclei from a younger sac.

Fig. 40. Embryo-sac after fertilization. The sexual nuclei are fusing in the egg-cell, and the endosperm formation has begun.

Fig. 41. Fertilized egg of Fig. 40, more enlarged, showing conjugation of the sexual nuclei.

Fig. 42. Chalazal end of the same sac, more highly magnified, showing the enlarged antipodal cells and young endosperm.

Fig. 43. Chalazal end of an older embryo-sac.

Fig. 44. Egg-cell, with fusion of the sexual nuclei almost completed. One of the synergidae is clearly evident.

Figs. 45-47. Young embryos, *sus*, suspensor.

Fig. 48. Young embryo surrounded by endosperm.

Figs. 49-52. Median longitudinal sections of older embryos, showing the variation in form, and in the development of the suspensor.

Fig. 53. Three longitudinal sections of an older embryo; *a*, a nearly median section; *st*, stem-apex; *r*, root; *cot*, cotyledon.

Fig. 54. Nearly median section of the root portion of the same embryo as Fig. 53, more highly magnified.

Fig. 55. Central region of the same embryo showing the stem-apex, *st*.

Fig. 56. Longitudinal section of a nearly full-grown seed, showing the small embryo, *em*, and the large antipodals, *ant*; enlarged about eight times.

Fig. 57. The lower part of Fig. 56, more enlarged.

Fig. 58. Endosperm cells from a nearly ripe seed.

Fig. 59. Nucleus from one of the antipodal cells.

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