SYMBIOSIS IN FERN PROTHALLIA

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THE symbiotic associations so frequently met with in plants present one of the most interesting phenomena with which the biologist has to deal. While these associations are often not easily distinguishable from true parasitism, in many instances there is a genuine symbiotic relation and, although there may be a certain degree of parasitism, there is no question that these associations are for the most part beneficial to both of the forms concerned. Indeed, the very existence of one or both of the symbionts may depend upon it.

In most cases of symbiosis, one of the symbionts is a fungus, but this is not always so. Certain of the Schizophyceæ or blue green algæ are very commonly associated with higher plants in what appears to be a symbiotic relation, although the nature of the association in this case is still very obscure. The Anthocerotaceæ and several of the liverworts, like Blasia, always have within their tissues colonies of a Nostoc, and the little water fern Azolla invariably harbors in each leaf a colony of the Nostoc-like *Anabæna Azollæ*. Nostoc has been found to occur in the roots of *Cycas revoluta* and Gunnera among the seed plants, and the Schizophyceæ also frequently constitute the "gonidia" of many lichens. The association of the nitrogen-fixing bacteria with the root nodules of the Leguminosæ is also a well-known case of symbiosis. Of the true algæ there are a number of species recorded, *e. g., Chlorochytrium Lemnæ,* which live within the tissues of higher plants, but it is doubtful whether the host is in any way affected by the presence of the alga, which presumably secures lodging, but not board, from its host.

The symbiotic association of fungi with green plants was first demonstrated in the lichens, but it is now known that many of the higher plants are regularly associated with fungi in what is undoubtedly a symbiotic relation. The best known cases of these are the mycorhizal fungi connected with the roots of many trees, especially the Cupuliferæ, and those which occur in the tissues of saprophytes growing in humus. These humus saprophytes are especially numerous among the Orchidaceæ, *e. g.*, Neottia, Corallorhiza, Cephalanthera, etc., and in certain forms of the Ericales. The well-known Indian pipe, *Monotropa uniflora,* and the snow plant of the Sierra Nevada, *Sarcodes sanguinea,* are well known examples of these saprophytic Ericales. Many Orchidaceæ and Ericaceæ which possess chlorophyll are also to a greater or less extent saprophytic and show a well-developed mycorhiza. In the case of chlorophylless plants, there can be little doubt that the fungus enables them in some way to utilize carbonaceous compounds from humus. In the case of plants such as the trees referred to, where there is ample chlorophyll tissue, it is more likely that the rôle of the mycorhiza is rather to supply nitrogen than carbon, and it is highly probable that in the case of chlorophylless saprophytes as well, the fungus provides nitrogen. This has recently been demonstrated for the mycorhiza found in the roots of various Ericaceæ, *e. g.,* species of Erica, Vaccinium, Calluna and Oxycoccus[1] In all of these it was shown that the fungus concerned, which seemed to be a species of Phoma, was able to assimilate free nitrogen in much the same way as is done by such nitrogen bacteria as Azotobacter.

A very complete study of the endophytic fungi of roots has been made by Janse.[2] He examined a very large

[1] Dr. Charlotte Ternetz. Über die Assimilation des atmosphärischen Stickstoffes durch Pilze. *Pringsheim's Jahrbücher*, XLIV, July, 1907.

[2] Les Endophytes Radicaux de quelques Plantes Javanaises. *Ann. du Jardin Botanique de Buitenzorg,* XIV, 54–201, 1895.

number of plants, mostly phanerogams, but also a number of liverworts and pteridophytes. His researches showed the presence of an endophytic mycorhiza in a surprisingly large number of plants belonging to the most diverse families, from Zoopsis, one of the Hepaticæ, to Vernonia, a genus of Compositæ.

The study of the mycorhizal fungi of the seed plants has called attention to the presence of similar fungi in the pteridophytes. The occurrence of a mycorhiza in the roots of the Ophioglossaceæ was first shown by Russow.[3] In 1884 Treub described a similar fungus from the gametophyte of Lycopodium. In his very important paper on the prothallia of Lycopodium[4] he pointed out the universal occurrence of this fungus in *L. cernuum*, and in later papers he showed that this also occurred in *L. phlegmaria*, as well as in some other species, but it was apparently absent from the green prothallium of *L. salakense*. In 1895 I called attention to the presence of a similar endophytic fungus in the subterranean prothallium of *Botrychium virginianum*.

The past decade has been notable for the numerous important investigations upon the gametophytes of the Ophioglossaceæ and the Lycopodiaceæ and our knowledge of these is now quite complete, thanks to the labors of Bruchmann, Lang and Jeffrey. It is clear that in all prothallia of the subterranean, and hence purely saprophytic type, an endophytic fungus is invariably present. It has also been shown that a similar form occurs in the green prothallium of some species, at least, of Lycopodium; but so far as I am aware its occurrence in the green prothallium of ferns has not hitherto been recorded.

Some time ago, having occasion to look over slides of the prothallium and embryo of *Osmunda cinnamomea*, it was noted that many of the prothallia contained an endophytic fungus very similar to that found in Botrychium

[3] Vergleichende Untersuchungen der Leitbündelkryptogamen. *Mem. de l'Akad. Imp. des Sc. de Petersbourg*, 1872, XIX, 107–118.

[4] Etudes sur les Lycopodiacées. *Ann. du Jardin Botanique de Buitenzorg*, IV, 1884.

and Ophioglossum. This suggested the possibility of its occurrence in other green prothallia, and the forms which seemed to promise best were the Marattiaceæ, which in many ways seem to be the nearest relatives of the Ophioglossaceæ, in whose subterranean prothallia the endophyte regularly occurs. I therefore made an effort to obtain prothallia of the Marattiaceæ while collecting in Ceylon and Java, and procured prothallia of *Angiopteris evecta* Hoffm, *Kaulfussia æsculifolia* Bl., and *Marattia sambucina* Bl. The two former were carefully studied, and as was expected, the endophyte was found in nearly every case. The prothallia of *Marattia sambucina* were not examined, but the examination of a series of sections of *M. Douglasii* Baker, made some years ago, showed that in this species the endophyte was also present and presumably it occurs also in other species of Marattia.

The other family of ferns in which it was thought the endophyte might occur was the Gleicheniaceæ, a small family, mostly tropical and of wide distribution. The Gleicheniaceæ are considered to be related to the Osmundaceæ and it was thought that they also might show the presence of the endophyte. The prothallia have rarely been collected, but are not difficult to find if one looks for them carefully. Material of four species was secured, one being collected near Cape Town, the others in Ceylon and Java. In all cases the endophytic fungus was found in the older prothallia.

These investigations show conclusively that an endophytic fungus is normally present in the green prothallia of several Marattiaceæ, Osmundaceæ and Gleicheniaceæ, and it is highly probable that further research will show similar fungal endophytes occurring in the prothallia of many other ferns.

The Structure of the Endophyte

Since the discovery of the endophytic fungus in the gametophyte of Botrychium, it has been found constantly in all the investigated species of Ophioglossaceæ, and it is safe to assume that it is invariably present and is essential to the growth of the gametophyte.

The writer has recently had occasion to study the behavior of this endophyte in the gametophyte of several species of Ophioglossum and has described and figured this somewhat at length.[5] The fungus consists of non-septate, large, branched hyphæ, which are strictly intracellular, passing from cell to cell through the cell walls, and they may often be traced for long distances. In all of the forms that have been investigated the fungus is confined to the older parts of the gametophyte, and never invades the meristematic tissues nor the tissues in the neighborhood of the young reproductive organs. There is in the cylindrical branches of the gametophyte of Ophioglossum a more or less definite infected zone inside the superficial tissues, while the central region remains almost entirely free from the endophyte. Sometimes fragments of mycelium are found upon the outside of the gametophyte, and these may occasionally be found to penetrate into the rhizoids and thus gain entrance to the inner tissues. The infection, however, probably in all cases takes place first while the gametophyte is still composed of very few cells. This was positively demonstrated in the germinating spores of *O. pendulum*, where only those young prothallia which succeeded in establishing a connection with the fungus developed beyond a three or four-celled stage. Otherwise they died after the nutrient matter in the spore was exhausted. Secondary infections, however, doubtless take place frequently. The form of the fungus growing outside of the prothallium is quite different from that within its tissues. The hyphæ in the former case are more slender and sometimes septa may be formed, while these seem to be quite absent from the endophytic hyphæ.

In material fixed with chromic acid, the structure of

[5] Campbell. Studies on the Ophioglossaceæ. *Ann. du Jardin Botanique de Buitenzorg*, XXVI, 1907.

the hyphæ is well shown. The walls, which in the ordinary hyphæ are moderately thick, stain well with gentian violet, while in the finer granular cytoplasm there are more or less numerous small bodies which stain strongly with safranin and are with little question nuclei. Some of the cells of the host contain unmodified hyphæ, which may be so numerous as to fill the cell cavity with a dense coil of filaments. In other cells the hyphæ form masses of irregular swollen vesicles with much more delicate walls than the ordinary hyphæ, and sometimes quite filling the cell. Besides the irregular vesicular swellings of the hyphæ described above, there may occur large oval or round structures (Fig. 1) which resemble the young oogonia of Pythium or Albugo. These may have a diameter of nearly 50 μ, but are usually smaller. The nuclei in these bodies are more numerous than in the vegetative hyphæ, and finally may be very conspicuous (Fig. 1, *C*). This multiplication of the nuclei resembles the preliminaries of zoospore formation in the sporangia of Saprolegnia or Pythium, and occasionally there were seen free in the host cells small bodies that looked as if they might have been discharged from these large oogonium-like bodies. The latter are probably identical[6] with

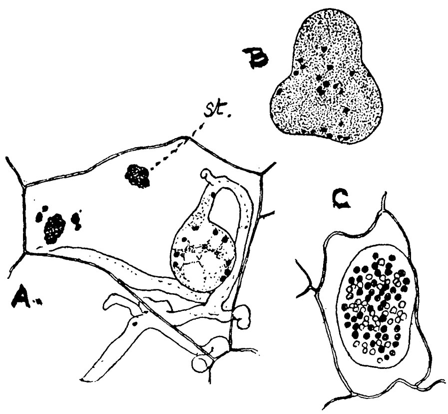

FIG. 1. *A*, Cell from the gametophyte of *Ophioglossum pendulum*, showing the mycelium of the endophyte, and a young conidium (?); *st*, masses of disintegrating starch granules; *B*, large conidium (?) of the same; *C*, fully developed conidium (?), showing the numerous enlarged nuclei; all × 350.

[6] Jeffrey. The Gametophyte of *Botrychium Virginianum*. *Proc. Canad. Institute*, V, 1898.

the "conidia" described by Jeffrey in Botrychium, but do not show the thick walls of these conidia. Like these conidia of Botrychium they are not, usually at least, separated from the filament by a septum. The young cells of the gametophyte contain starch in the form of rather small and very distinct granules. As the endophyte invades these cells, the starch granules soon show evidences of disintegration, swelling up and losing their sharp contour and finally becoming aggregated in irregular masses of considerable size (Fig. 1, *A*, *st*). These finally are more or less completely digested by the fungus, but the nucleus of the host cell is in no way affected, and even where the cell is completely filled with the crowded hyphæ, the nucleus remains quite normal in appearance.

The endophyte of *Botrychium virginianum* (Fig. 2) closely resembles that of Ophioglossum, but is somewhat smaller in all its parts and occupies the whole central region of the massive gametophyte. The two sorts of

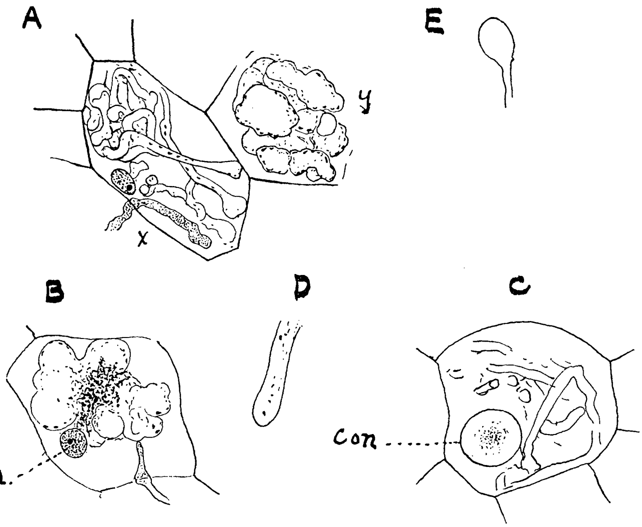

FIG. 2. *A*, two cells from the gametophyte of *Botrychium virginianum*, showing the two forms of the endophyte; *B*, a "digestive" cell, showing the degenerating varicose mycelium of the endophyte; *n*, the nucleus of the host cell; *C*, cell containing a conidium, con; *D*, fragment of one of the largest hyphæ; *E*, young conidium. All figures × 350.

cells, *i. e.*, those with the filamentous hyphæ (Fig. 2, *A*, *x*) and those containing the irregular vesicular mycelium, (*y*), are well differentiated, but are more or less irregularly mingled. The "conidia" (Fig. 2, *C*, *con*) are smaller and less numerous than in the endophyte of Ophioglossum, but have a much firmer membrane, as Jeffrey has described. These conidia were observed by Jeffrey to germinate by sending out a tube, and they are supposed to be special organs of propagation.

In a very important study of the endophytic mycorhiza of the saprophytic orchid, Neottia, W. Magnus[7] has shown that two types of mycelium exhibited by the endophytes are of very different nature. The slender cylindrical hyphæ constitute the active portion of the fungus, which behaves like a parasite toward the cell which it invades, destroying the starch and probably other constituents of the cell, but not attacking the nucleus. The latter becomes much hypertrophied, a phenomenon that is not seen in the endophyte of the Ophioglossaceæ. The swollen vesicular mycelium, however, is a degenerating structure and is itself destroyed by the cells of the host, which actually digest these fungus mycelia in much the same way that the cells of Drosera digest their prey. Some interesting similarities in the behavior of the contents of the digestive cells of Drosera and those of these humus saprophytes have been demonstrated. Figs. 2, *A* and *B*, show some of these cells in Botrychium; the varicose mycelium has very delicate walls, and in the older cells (Fig. 2, *B*) they seem to be disintegrating until finally the structure is completely destroyed and only a structureless lump is left. In Neottia this undigested mass is ejected into a central vacuole and becomes surrounded with a more or less evident cellulose membrane, separating it entirely from the protoplast after digestion is complete.

A comparison of the endophytes found in the green

[7] Studien an der Endotrophen Mycorhiza von Neottia Nidus Avis L. *Pringsh. Jahrb.*, XXXV, 1900.

prothallia of the various ferns referred to shows some differences which are probably not without significance. The structure of the mycelium and its general behavior are so much like those of the endophyte occurring in the strictly saprophytic gametophyte of the Ophioglossaceæ as to leave little doubt that the endophyte in each case

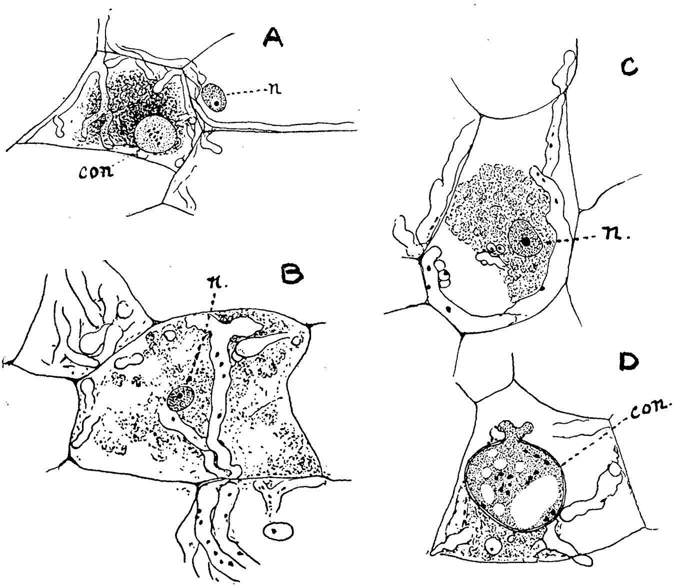

FIG. 3. Cells from the green gametophytes of several ferns, showing the character of the endophyte. All figures × 350.
A, Angiopteris evecta; B, Osmunda cinnamomea; C, D, Gleichenia pectinata.

is the same, or at any rate closely related. The conidia (Fig. 3, *A, C*) are perhaps less frequent, but in form and structure are very like those of Botrychium. The most noticeable difference is the apparently complete absence of the "digestive" cells, *i. e.*, those that contain the varicose swollen mycelium. No indications were noted of the destruction of the fungus by the cells of the host and the former is evidently much more nearly a true parasite than is the case in the saprophytic gametophytes. In the infested cells of the green gametophyte the starch and chromatophores are destroyed evidently by the action of the endophyte, but the nucleus remains intact.

Of the ferns with green prothallia, the endophyte has been found, almost without exception, in the following: *Marattia Douglasii* Baker, *Kaulfussia æsculifolia* Bl., *Angiopteris evecta* Hoffm., *Gleichenia* (*Eugleichenia*) *polypodioides* Sm., *G.* (*Mertensia*) *dichotoma* Willd. (=*G. linearis* (Burm.) Bedd), *G.* (*Mertensia*) *lævigata* Hooker, *G.* (*Mertensia*) *pectinata* Presl. In *Osmunda cinnamomea* it appears to be commonly but not always present, and in *O. Claytoniana* it could not be found. The number of slides of the last species examined was not very large and it is possible that further study of this species, as well as of *O. regalis,* will show its further occurrence in the Osmundaceæ.

Of the forms that were examined, that occurring in Osmunda and Gleichenia was the largest (Fig. 3, *B, C*) and equal in size to the endophyte of Ophioglossum. The form in Angiopteris was the smallest that was seen.

The Significance of the Endophyte

That the presence of the endophyte is necessary to the existence of all strictly saprophytic gametophytes is indicated by the failure of the germinating spores to develop unless they become associated with the fungus. Moreover, the universal occurrence of a similar endophyte in all humus-saprophytes among the seed plants indicates that in all of these chlorophylless plants the presence of the fungus is necessary for the existence of the host. Although it has not been directly proved, it is generally assumed that one rôle of the endophyte is the elaboration of some of the carbonaceous constituents of the humus. The infrequent communication between the external hyphæ and the internal mycelium makes it unlikely that the nutritive products are directly absorbed by the fungus, and it seems much more probable that the rhizoids of the gametophyte are the direct agents of absorption. How the humus constituents are changed by the action of the fungus so that they are available for the cells of the host is not clear and it is by no means impossible that some at least of the necessary carbon may be derived from the fungus itself in the digestive process to which it is subjected in the digestive cells. This seems plausible from the fact that in green prothallia, where presumably the plant is entirely able to supply its own carbon compounds through photosynthesis, these digestive cells appear to be wanting; or at any rate they were not observed in the several forms that I have studied. The experiments of Ternetz already referred to showing that certain fungi, including certain endophytic mycorhizæ, have the ability to assimilate free nitrogen, confirms the assumption of earlier authors that the fungus is useful to the host in supplying to it nitrogen compounds; but while this is probably a very important part of its functions, it seems to me that it is not perhaps the only one, and that the necessary carbon is also supplied directly or indirectly through the agency of the fungus.

As Magnus has very graphically shown, the relation of the two symbionts is mutually antagonistic, each one acting as a parasite on the other, but nevertheless the presence of the fungus is essential to the higher organism so long as the latter is destitute of chlorophyl; and the explanation of the wide-spread saprophytism exhibited by so many of the higher plants may be sought in this attempt to defend themselves against what was probably at first a strictly parasitic organism. Having acquired the power to attack and feed upon the parasite, the photosynthetic functions were more and more subordinated until a state of true parasitism (or saprophytism) resulted. The numerous semi-saprophytes like most of the green Ericaceæ and many green Orchidaceæ are good examples of transition stages, while the characteristic leafless humus saprophytes, such as the Monotropeæ and the chlorophylless Orchidaceæ, represent the fully developed phase of this peculiar form of symbiosis.

That this symbiotic association may occur in still lower organisms than the ferns is shown in the familiar case of the lichens, which are most perfect examples of this. It has been shown also that a similar association of fungus and host occurs in a good many liverworts. Cavers[8] has studied this association with some care in the common liverwort Fegatella, as well as in other Hepaticæ. He found in Fegatella that the endophyte is beneficial to the growth of the host, the plant being more vigorous when the fungus was present. He assumed that this was due to the assistance given by the fungus in the assimilation of organic matter from humus or from other organic substrata.

This frequent occurrence of an endophyte in Hepaticæ makes the occurrence of this in the green prothallia of ferns quite readily understood. Whether in the latter it is an advantage to the host to have the endophyte present remains to be seen, but it is highly probable that such is the case. Once having acquired the habit of associating itself with the fungus, the gradual development of the purely saprophytic subterranean gametophytes of the Ophioglossaceæ from green forms similar to those of the Marattiaceæ, is readily conceivable. In the genus Lycopodium there is every degree from the strictly holophytic green prothallium of *L. salakense* to the subterranean chlorophylless gametophyte of *L. clavatum* or the still more specialized gametophyte of *L. phlegmaria*.

Presumably in the Ophioglossaceæ the evolution of the gametophyte has been very much the same as in Lycopodium.

[8] On Saprophytism and Mycorhiza in Hepaticæ. *New Phytologist*, II, 1903.