

ART. XXXVII.—On the Prothallium of *Phylloglossum*.

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OUR knowledge of the development of the isosporous *Lycopodium* is still so incomplete that no apology seems necessary for the present preliminary statement. A special interest attaches to *Phylloglossum* since it has been recognised as a permanently embryonic form of lycopod.† *Phylloglossum* is a genus with a single species—*P. drummondii*—confined to Australia and New Zealand. The sporophyte generation is a small plant growing from a tuber, which forms a tuft of a few cylindrical tapering leaves. In fertile plants the apex of the stem forms a peduncle, terminated by a cone or strobilus of small scale-like fertile leaves.

Treub has shown that the embryo of *L. cernuum* shows a remarkable likeness to a barren plant of *Phylloglossum*, for the first formed leaves have the character of the leaves of *Phylloglossum*; hence the term "protophylls" has been given to these structures, so different from the ordinary leaves of lycopods. Further, the embryo of *L. cernuum* forms at a very early stage a tuber (protocorm), above which its protophylls rise. To this protocorm the tuber of *Phylloglossum* is apparently comparable, but in *Phylloglossum* it is not a passing embryonic structure, but is repeated annually on the formation of a new protocorm. Treub regarded the protocorm as the representative of a primitive structure originally possessed by the pteridophytes, a structure which may have served an important part in the phylogeny of the higher plants, in enabling the sporophyte to attain an existence independent of the gametophyte.

The prothallia of *Phylloglossum* have been obtained growing naturally amongst the parent plants, but it is a significant fact that in most places, even where older plants are abundant enough, no prothallia could be discovered, though many days were spent in fruitless search. In three localities only were prothallia discoverable. It appears clear that very special conditions are necessary for the germination of the spores, conditions which are not of regular annual occurrence wherever *Phylloglossum* grows. Perhaps the most important of these conditions is the presence of a fungus with which the

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† Bower, Phil. Trans., 1886, p. 676.

prothallium lives symbiotically. Such a symbiotic fungus has been found in the prothallia of almost all the species of *Lycopodium* in which the development is known.

Before describing the prothallia it will be well to state that they vary remarkably in external form. Such variations as depend upon the stage of development present no difficulty, but there are other differences which are probably accidental, being due to obstacles in the soil or to the depth beneath the surface at which the prothallium commenced its development.

One of the simplest, and perhaps the youngest observed, consisted of an oval tuber below, from which rose a simple cylindrical shaft with rounded apex. Such a prothallium appears to closely correspond with the oldest prothallium of *L. cernuum* obtained in laboratory cultures by Treub. We may take the tuber, which is of constant occurrence, to correspond to the primary tubercle seen in the prothallium of *L. cernuum*.

A more advanced prothallium shows the cylindrical part of greater length and thickness, and its end slightly expanded into a crown, on which the first sexual organs appear. A little below the crown the tissues of the cylindrical body are conspicuously meristematic, especially on one side. This meristem lies below the archegonia, and its formation appears to be anticipatory of the descent of the embryo.

Older prothallia in which an embryo is already present are much more irregular in form. The crown, which may be conical, rounded, or projecting to one side, and then often shaped like the head of a horse, is commonly separated by a slight constriction from the much-enlarged part of the body of the prothallium. This latter part bears the embryo on one side; it is evidently formed by the increase of assimilatory tissues for the nutrition of the embryo. Below this swollen part the body contracts again to a cylindrical shaft, which passes downwards, to swell out again and terminate in the primary tubercle. It is this shaft which varies most; it may be long and narrow, straight or curved, or it may be shorter and stouter, or occasionally, perhaps when the primary tubercle has been formed near the surface of the ground, it may be almost obsolete. The shorter thick-set prothallia may be less than 2 mm. long; others may range up to thrice this, according to the length of the shaft. Rhizoids are produced in considerable numbers from the lower part of the prothallium, more especially from the tubercle.

The whole of the upper part of the mature prothallium is green, except the archegonial necks, but the minute chloroplasts are most abundant in the part below the crown. The cells of the shaft may contain chloroplasts, but the green colour passes away as we follow it downwards into the soil.

Sections of the prothallia show little internal differentiation of the tissues, certainly nothing which is comparable to that described in *L. complanatum* and *L. clavatum*. The cells of the tubercle appear of a rounded polygonal form. They show scanty protoplasmic contents, and appear partly exhausted. Those of the shaft are elongated; on the surface they are rectangular, in the centre they tend to become longer and more pointed. Starch is often abundantly present in the cells, especially of the central part. An endophytic fungus may be traced in the cells of the lower half of the prothallium. The hyphæ are exceedingly fine; they have been traced passing in through the rhizoids. Around the tubercle they often form a close felt, which may pass below into a strand, which suggests at first sight that the base of the tubercle passes into a root. The tubercle is commonly brownish on the surface, and the strand is a darker brown and almost opaque. But sections show that it consists of fungus hyphæ.

The prothallia are monœcious, and the archegonial necks are a conspicuous feature on the crown. On a young prothallium I have found two or three only, but on plants bearing an embryo there may be from ten to twenty. They appear to be formed in basipetal succession. In a young prothallium they may be found on the summit of the crown, but in older ones they seem to occupy a lateral position around about half the circumference. The neck of the archegonium projects from the surface of the crown as a hemisphere of colourless cells, usually in two tiers of four cells each. The venter, with the large oosphere, lies at a little depth below the surface. The antheridia can scarcely be said to show in surface views, as they lie sunk in the crown. Sections show that the antheridial cavity is elongated at right angles to the neighbouring surface. The cover-cells form a single layer. The sexual organs would seem to resemble those of *L. cernuum* more closely than those of other species of *Lycopodium*. There are no multicellular paraphyses amongst the sexual organs as in *L. selago* and *L. phlegmaria*, but on some parts of the crown the surface-cells are slightly papillose.

The thickness of the tissues renders it impossible to follow the details of the early development of the embryo, except in microtome sections. But it seems clear from the stages which have so far been examined that the development at first is much like that of the embryo in *L. cernuum*. The embryo grows obliquely downwards and outwards; the part near the archegonial venter is the foot, at the opposite end are formed the stem-apex and leaf. The first part of the embryo to appear outside the prothallium is the tip of the leaf; it breaks out at a point on the side of the thicker part of the prothallium, below the crown. A fissure extends thence

down the side of the prothallium, and the embryo appears as a short cylindrical body, bluntly pointed at both ends, placed vertically, and still connected with the prothallium by the foot, which now has a lateral position. The ends of the embryo grow downwards and upwards respectively, and at a later stage what is really the apex of the stem appears inside the lower part of the embryo—that is, the embryo immediately on escaping from the prothallium forms a protocorm, apparently in the same manner that the adult plant forms its annual tuber. The pedicel of the tuber elongates downwards until the latter is placed at a safe depth, about 3 mm., in the soil. In the meantime the leaf grows up and attains a height of 2 mm. to 5 mm. above the ground. I have not hitherto seen any formation of root during the first year of growth, the sporophyte seemingly depending largely for its supply of moisture upon the prothallium, which sometimes retains its vitality even after its crown becomes injured by drought. But sometimes, at any rate, rhizoids may be developed on the pedicel and protocorm. The leaf becomes green even before it escapes from the prothallium, and as soon as it reaches a little above the soil stomata are formed, and air passes into the intercellular spaces, whilst a slender strand of tracheids appears in the centre. The first prophyll has, in fact, exactly the structure of a small leaf as produced in later years. The further development of the sporophyte appears to be slow. By carefully dissecting out the plants in the soil one can find the remains of tubers and roots produced in former years. In many cases the plant comes up a second and a third year with only a single leaf.

It should be mentioned here that Crié has stated that he sowed the spores of *Phylloglossum* and obtained a colourless prothallium like that of *Ophioglossum*. But his statements have not been accepted, and recent writers, as Vines, Bower, Campbell, Goebel, Pritzel,* distinctly state that the development of *Phylloglossum* is not known. I have not had access to Crié's original account, and Bertrand,† who quotes Crié's statements, was unable to obtain a germination of the spores during six years' experiments. But in any case it is clear that Crié's account was incomplete, for the prothallium becomes green, and even vividly green. According to Bertrand, Crié can only have seen the tubercle which precedes the prothallium proper.

It is not improbable that the prothallium may start life as a saprophyte, aided by the endophytic fungus, and I have

* *E.g.*, Goebel, "Organographie der Pflanzen," 2te Teil, 1900, p. 439. Pritzel, in Engler and Prantl "Die Natürlichen Pflanzen-familien," Lief. 205 (1900), p. 575.

† "Archives Botaniques du Nord de la France," 1886, p. 221.

found a young prothallium which was quite colourless save for a faint yellow tinge at the upper end, as well as two others, still without sexual organs, which showed only scanty chloroplasts. It is quite probable that on the germination of the spore the tubercle is first formed, and when this is at too great a depth in the soil to receive any light it will doubtless be colourless. But I have never observed any fully developed prothallium that was not green above, whilst all prothallia which had succeeded in producing an embryo had reached the surface and attained a considerable development of chlorophyll.

A comparison of the prothallium of *Phylloglossum* with those of the few species of *Lycopodium* in which the gametophyte is known shows that it is distinctly of a Lycopod type. But, as is well known, there is a remarkable diversity amongst the prothallia of the different species of *Lycopodium*. On the whole, the prothallium of *Phylloglossum* probably resembles a prothallium of the *L. cernuum* type more closely than any other, though it is quite without the leaf-like assimilatory lobes of *L. cernuum*. Perhaps we are justified in regarding it as the simplest known type amongst the isosporous *Lycopodinae*.

The general simplicity of the structure of the prothallium of *Phylloglossum* seems to favour the view that it is a primitive form of lycopod. It is, of course, recognised that *Phylloglossum* is a permanent embryonic form, but the simplicity of structure of the mature sporophyte does not necessarily prove that it is a primitive form of the lycopodiaceous phylum. Bower has expressed the view that *Phylloglossum* is probably a reduced form, and the absence of transitions between the simple cylindrical pointed protophylls and the scale-like sporophylls, so like those of some species of *Lycopodium*, may favour this view, if we regard these structures as homologous. Some observations which appear to be new may throw some light upon this question. Bower states that *Phylloglossum* has been seen branched. I am able to say that branching occurs in at least two distinct ways:—

1. The spike or strobilus occasionally branches; perhaps one strobilus in two thousand will be found forked, the two divisions becoming equally developed. I am, of course, only speaking of the form which grows in New Zealand, and this may possibly be a slightly more robust form than that found in Australia. The branching usually takes place above the lowest sporophyll, sometimes quite at the base of the spike, near the lowest leaf, sometimes further up, or even close to the apex of the strobilus.

But even when the strobilus forks there is no transition of form between the sporophyll and protophyll. I have occasion-

ally observed on the peduncle a leaf some distance below the rest of the strobilus, but such a leaf has always been of the sporophyll type. In the Australian form, investigated respectively by Bower and Bertrand, to whom we are indebted for most of our knowledge of *Phylloglossum*, eight was the largest number of protophylls found on a plant, whilst Bertrand urges on anatomical grounds that six is the normal number of protophylls. I have collected plants with twenty protophylls, whilst others with ten to fifteen such leaves are of common occurrence. But even in plants richest in protophylls no transition occurs between protophylls and sporophylls. So far as any evidence here available goes, it would almost seem as if the two structures were not strictly homologous.

To express my meaning in the language of a modern theory, the protophylls may have arisen from the differentiation of the lower region of a sporogonium (or the homologue of a sporogonium) in which this region had already acquired sterilised tissues, whilst the sporophylls arose from the upper fertile region of the sporogonium. If so, the protophylls cannot be regarded as sterilised sporophylls.

There appears to be no necessary connection between the number of protophylls and the reproduction by spores. Plants with two protophylls only may produce a weak spike, whilst plants of twenty protophylls may be barren.

2. In barren plants the new tuber is formed by the lowering of the apex of the stem, but in fertile plants a new outgrowth is formed, which Bower regards as adventitious. This may doubtless be considered as a form of branching. Neither Bertrand nor Bower observed more than a single tuber formed in the examples at their disposal. Bower, indeed, was inclined to infer that, as no other mode of vegetative reproduction was known, the plant depended for its multiplication solely upon the germination of the spores. But I have found that the formation of two new tubers is quite a common occurrence, though plants which form a single tuber are still in the majority. The two new tubers may be formed on opposite sides of the plant, in which case a slight dispersion of the plants takes place. Sometimes the two tubers arise close together. Apparently they may be formed almost simultaneously, or in succession. Naturally, it is the stronger plants which most frequently multiply thus, but plants of a smaller number of protophylls may branch in this way. One plant of a single protophyll was found with two tubers forming.

The occasional occurrence of branching in the strobilus might be interpreted as an indication that the ancestors of the plant were once more abundantly branched. But it

would be possible to take the opposite view, that such branching is a nascent feature, that it is a new feature in the phylogeny. Bertrand regarded *Phylloglossum* as a form reduced on account of its semi-aquatic mode of life. But it is necessary to point out that *Phylloglossum* is not a semi-aquatic; Bertrand never had the advantage of seeing the plant in its native home. *Phylloglossum*, it is true, being a very small plant, can only grow whilst the surface soil is fairly moist, hence it forms a tuber and rests during the dry season. So far as I have seen, the plant grows rather better on a hill-top; or, at any rate, it grows there at least as well as it does lower down on the slope, and I have never found it in an actual swamp. It grows well on a slope where water can never lodge. Its roots spread rather horizontally, and seldom far downwards in the ground, as though it objected to a waterlogged soil.

Whilst it is possible that evidence may yet be adduced that *Phylloglossum* in some measure owes its simplicity to reduction, there appears to be little evidence for this at present. On the other hand, it may yet prove that *Phylloglossum* is an exceedingly primitive plant, possibly the most primitive of existing pteridophytes. We have an explanation ready to hand of this exceptional retention of ancient characters—namely, the annual renewal of the embryonic stage in the formation of the protocorm. But, however this may be decided, the relatively simple character of the gametophyte and the comparison of the mature sporophyte with the embryo of *Lycopodium cernuum* are in favour of the view that *Phylloglossum* is the most primitive of existing *Lycopodiæ*.
