NOTES.

THE GAMETOPHYTE OF BOTRYCHIUM VIRGINIANUM.—At the suggestion of Dr. Scott, the following note on the gametophyte of *Botrychium virginianum* has been prepared by the writer.

The account is slightly modified from an abstract published in the proceedings of the Canadian Institute, Vol. i, Part I, 1897. A full description with the necessary plates and references to the literature will shortly appear in the Transactions of the Canadian Institute.

A complete description of the gametophyte of the Ophioglossaceae has long been a desideratum. Since the discovery by Mettenius, in 1856, of the subterranean prothallium of *Ophioglossum pedunculosum*, and by Hofmeister, in 1857, of that of *Botrychium Lunaria*, nothing has been added till recently to their necessarily incomplete accounts of the gametophyte in these species. Our latest knowledge on this subject is derived from a brief description of rather advanced material of the prothallium of *Botrychium virginianum* found in 1893 at Grosse Isle, Michigan, by Professor Douglas Campbell, which was published in the proceedings of the Oxford meeting of the British Association in 1894.

A more extended description of the same material, together with an account of the first stages in the germination of the spores of *B. virginianum* and *Ophioglossum*, appeared in his 'Development of Mosses and Ferns' (1895). These prothallia did not, however, supply the stages in the development of the sexual organs and the sporophyte.

During the summer of 1895, the writer secured a large number of prothallia of the same species at Little Metis in the Province of

Quebec. On examination it was found that the material thus obtained afforded a complete elucidation of the development and structure of the antheridia and archegonia, and a less satisfactory series of stages in the segmentation of the embryo. Last summer the remaining prothallia were removed, to the number of about six hundred, and although they have only been partially studied yet, owing to technical difficulties in embedding them, those examined have supplied all the lacking stages of the development of the young sporophyte.

All the younger prothallia were found in a single circular depression of Sphagnum-moss about ten feet in diameter, near a corduroy road, running through the wooded margin of a peat and huckleberry swamp at Little Metis, P. Q. Older prothallia were abundant with those bearing fertilized and unfertilized archegonia and younger embryos.

I have also found young sporophytes of several years' growth in the woods on the heights back of Metis; in the 'Flats' below the 'Whirlpool' on the Niagara river, and also in rich woods along the valley of the Don, near Toronto. In all the examples last referred to the young spore-plant was still attached to the gametophyte. It seems probable that the prothallia of our common Canadian species of *Botrychium* are much more easily obtainable than has been hitherto supposed. It is necessary to add, however, that although my attention has been directed to the subject for some three years past, I have not yet succeeded in finding the younger stages of the prothallia in any other spot than the Sphagnum-basin in the swamp at Little Metis.

The gametophyte of *Botrychium virginianum* is of flattened oval shape, the narrower end of the prothallium being terminated by the growing-point. My specimens are from two to eighteen millimetres in length, by one and a half to eight millimetres in breadth. Their thickness increases from the growing apex backwards. The sides and lower surface of the prothallium are covered in younger specimens with multicellular hairs. In older plants these tend to disappear. The middle of the upper surface is occupied by a well-defined ridge, upon which the antheridia are situated. The archegonia are found on the declivities which slope away from the antheridial ridge.

As might be expected, the younger sexual organs are found nearer the growing-point than those of greater age.

A cross-section of the prothallium reveals to the naked eye the

fact that the lower part of the gametophyte is composed of tissue which is yellowish in colour, and from which a thick oil exudes, even when the plant has been lying in ninety per cent. alcohol for months. The upper portion of the prothallium-tissue, upon which the generative organs are situated, is white in colour and free from oil. A longitudinal section of the prothallium shows the same distribution of yellow oil-bearing and white oil-free tissue as the cross-section, but demonstrates that the oil-bearing stratum is both absolutely and relatively much thicker in the older parts of the plant.

Microscopic examination shows that the oleiferous tissue has its cells occupied by an endophytic Fungus and a very abundant protoplasm.

The Fungus, so far as it has yet been studied, seems to be a sterile *Pythium*, possibly the same as that found by Treub, Goebel and others, in the prothallium of species of *Lycopodium*. The writer hopes to investigate the Fungus more closely in a living condition during the next period of vegetation. The Fungus-filaments can be seen passing from the prothallium to the outside medium by way of the root-hairs.

The prothallium seems to be entirely saprophytic in its mode of life; as quite young examples, bearing as yet only antheridia, were found, which were nevertheless yellow in colour and wholly subterranean. They showed no evidence of a scar, indicating a possible origin from a green subaerial phase, even when examined under considerable magnification; and in fact the depth of their occurrence in the moss (in many cases 10 cm. or more) would seem in itself to preclude such a mode of origin. Moreover, Mettenius found in the case of *Ophioglossum pedunculosum*, that the subterranean saprophytic stage was antecedent to the green lobes, appearing above the soil. Somewhat similar conditions have been described by Treub in species of *Lycopodium*.

Campbell describes the appearance of chlorophyll in the germinating spores of this species, but it may have originated from the spores being sown, contrary to the natural conditions, in the light. The writer is experimenting with growing spores in darkness, but sufficient time has not yet elapsed for germination to take place.

The antheridia, as has been already stated, occur in numbers on a ridge running lengthwise on the upper surface of the prothallium. The young antheridia originate behind the growing-point from

a single superficial cell. This divides transversely, the outer half giving rise to the outer antheridial wall and the inner half by repeated simultaneous divisions to a large number of spermatocytes. The fully developed antheridium is largely embedded in the antheridial ridge, and projects only slightly above its surface. The formation of the spermatozoids has not yet been carefully studied, but seems to resemble closely that described in the Marattiaceae and Equisetaceae. The spermatozoids are usually large in size, but otherwise resemble the ordinary Fern-type, and consequently differ from the biciliate Moss-like spermatozoids of the Lycopodiales.

The archegonia are confined to the sloping sides of the upper surface of the prothallium. Unlike the antheridia, young archegonia, although most abundant near the growing-point, may be formed on almost any part of the archegonia-bearing surface. The mother-cell of the archegonium is superficial, and is distinguished from its neighbours by a large nucleus and a more abundant protoplasm. It first divides transversely into a shallow outer cell and a deeper inner cell. The inner cell divides again, and as a result the young archegonium consists of three cells. The most external of these, by subsequent divisions, give rise to the neck of the archegonium. The internal cell is the basal cell. It also divides into a plate of cells, sometimes composed of two layers, distinguished by their richly protoplasmic contents. The middle cell of the young archegonium-series gives rise by division to the neck-canal-cell and to the ventral cell. The former becomes binucleate, but never divides into two cells. The latter, just before the maturation of the archegonium, divides into the egg-cell and the ventral canal-cell. The ventral canal-cell is broad, like that of the Marattiaceae.

In the ripe archegonium, the nuclei of the cells of the upper stories of the archegonium-neck become chromatolysed. I do not know yet whether this feature is peculiar to *Botrychium*.

The fully-developed archegonium is sunk into the prothallium, and only the neck projects above its surface. The cervical cells are in four rows as in the other Pteridophyta, and the terminal ones spring apart when the egg is ripe.

Spermatozoids are frequently found in contact with the egg. After fertilization the egg grows to many times its original size, and the reduced protoplasm contains a large hydroplastid.

The first division of the oospore is across the long axis of the

archegonium. The next division is parallel with the long axis of the prothallium, and at right angles to the first. The third cross-wall is in the transverse direction of the prothallium, and at right angles to the other two. I have been unable to follow satisfactorily the subsequent divisions. The organs appear very late, and only after the embryo has attained a large size. The root is the first of them to emerge, and the proliferation of the cells, indicating its place of origin, is long unmarked by the presence of an apical cell. The cotyledon, stem-apex, and the foot, appear nearly simultaneously. The root and cotyledon originate from the upper part of the embryonic mass; the foot and stem-apex from its lower cells. The apex of the root in many cases is in the same straight line with the canal of the archegonium-neck.

It seems hardly possible to derive the organs from definite octants of the embryo.

The growth of the root ruptures the calyptra, and its exit is followed somewhat later by that of the cotyledon. The latter is not a bilaterally symmetrical structure, as in most Ferns, but is of the same palmate type as is found in the Osmundaceae. The cotyledon begins to assimilate as soon as it reaches the surface of the ground, and thus resembles that of *Ophioglossum pedunculosum*.

There seems to be no evidence to indicate that more than the cotyledon appears above ground in the first season of the young plant's growth. In following summers apparently only a single leaf is produced, as is the case with the older plant. I have found young sporophytes, bearing their sixth leaf, still attached to the mother-prothallium; and as I have never found more than one leaf on the young spore-plants at once, and as the leaves, like other organs of this species of *Botrychium*, are extremely resistant to decay, I am reasonably certain that such examples were in the sixth year of their existence. This longevity of the gametophyte is of some interest.

One frequently finds two or more sporophytes on a single prothallium, and in many of these cases the apex of the prothallium is bifurcated. In one case I found two spore-plants which had arisen from a single embryo. In another case I discovered two tracheids in a prothallium in the vicinity of a decayed young spore-plant. The latter may have been of apogamous origin, as a similar phenomenon generally accompanies apogamy. I have not yet studied thoroughly the growing region of the prothallium, as it is best examined in

longitudinal sections of the gametophyte. So far as I have investigated the matter, there seems to be evidence of the existence of an apical cell.

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BACTERIA WITH ASSIMILATORY PIGMENTS, FOUND IN THE TROPICS.—The following Bacteria, having a greenish colouration and showing when exposed to light a faint evolution of oxygen, perceptible by means of motile Spirilla or Micrococci and very exceptionally causing *Bacterium termo* to faintly re-act, were found in water-cultures of more or less purity developed in diffuse daylight at Buitenzorg: viz. a motile green Bacterium=*B. chlorinum* (Engelmann)[1]; a non-motile Micrococcus-form to which the provisional name of *Streptococcus varians*[2] has previously been given; two forms closely resembling Van Tieghem's *Bacillus virens* and *Bacterium viride*[2]; two green Spirilla, one resembling *S. tenue* and the other *S. undula*; and finally a large Bacillus-form somewhat resembling the *Bacillus virens* of Van Tieghem. This last form occurs as short rods, 2·5 to 3 μ broad and commonly 12 to 15 μ, more rarely 5 to 20 μ long. A formation of colourless refractile endosporous spores is often shown. The spores are oval and 1·5 to 2 μ broad by 2 to 3·5 μ long. In all cases the pigment is diffused throughout the plasma of the bacteroid-cell, and this is especially clearly shown in the large *Bacillus virens* and *Spirillum undula* forms.

Of the two more common red water-Bacteria, *Monas okenii* was not found in either Java or Ceylon, but *Bacterium photometricum* was. In Java *B. photometricum* appears to be abundant and widely distributed. In water-cultures exposed to diffuse daylight, the Bacteria collect in the form of a red crust upon the walls of the cylinder. By removing this crust a large mass of the red *Bacteria* may be obtained; which if the growth is of recent formation may be nearly pure, and, what is of more importance, almost entirely free from other coloured Bacteria or Confervae. If to the brownish red mass thus obtained alcohol is added, the resulting fluid is reddish in colour, turning to a dark dirty green on warming. If ether is now added

[1] Engelmann, Zur Biologie der Schizomyceten, Bot. Zeit. 1882.

[2] The Evolution of Oxygen by coloured Bacteria: Journal of the Linnean Society, 1897.

[3] Van Tieghem, Bull. Soc. France, XXVII, 1880, p. 174.