The Spermatogenesis of *Polytrichum juniperinum*.

BY

CHARLES E. ALLEN.

With Plates XV and XVI.

INTRODUCTION.

IN a previous paper (Allen, 1912) I have followed the history of the successive generations of cells that occupy the interior of the antheridium of *Polytrichum juniperinum* down to the final division—that, namely, of the androcyte mother-cells. The present study is concerned with the metamorphosis into antherozoids of the androcytes formed by this division. Certain of the observations here to be described have already been briefly reported (Allen, 1908, 1913, 1914).

The methods used in this work were in the main those described in my paper of 1912. For the study of the fully developed antherozoids, the mass oozing from a mature antheridium into a drop of water was exposed for a minute or two to the fumes of osmic acid, or, in most cases, the slide bearing the drop was inverted over a small wide-mouthed bottle containing medium or strong Flemming's solution. After fixation by this means, the slide was allowed to dry thoroughly, drying sometimes being hastened by a brief heating over a flame. Then the slide was immersed in the stain or stains to be used, thoroughly dried again, a drop of clove oil or other clearing agent was placed upon the material, and this was followed by Canada balsam. Dehydration of the stained preparations by alcohol was avoided after a few experiences, since it was found that the alcohol quickly extracts all colour from the cilia. Numerous stains and combinations of stains were used for the antherozoids so prepared; the best results were obtained with acid fuchsin and iodine blue, safranin and gentian violet, and safranin and pyoktanin blue.

OBSERVATIONS.

A description has previously been given (Allen, 1912) of the small darkly staining body present in each young androcyte. This dense granule seems to be identical with that which behaved like a central body in the last preceding mitosis; in the development of the androcyte into an

antherozoid it is destined, as will be seen, to function as a blepharoplast. When a pair of sister androcytes can be recognized as such (as in Figs. 1 and 2, Pl. XV), the blepharoplast in each is usually to be found in a position corresponding to that of a central body in the division recently completed. The blepharoplast is sometimes (Figs. 1-3) the centre of a system consisting of a few radiations much like those that surrounded the single central body which was found previous to the initiation of mitosis in each androcyte mother-cell. The cytoplasm immediately about the blepharoplast is commonly somewhat more dense than that in other parts of the cell. The cytoplasmic contraction and rounding up, which began in the androcyte mother-cells, have now gone on to a considerable extent. The walls (excepting those laid down in the course of the last division) are much thickened and show evidence of softening. The chromatic substances within the nucleus still exhibit more or less of the forms of the chromosomes; in some cells (Fig. 2) there is often no body as yet recognizable as a nucleole. It was pointed out in the paper just cited that nucleoles make their appearance much later in the androcyte nuclei than they do in the daughter nuclei formed by any of the androgonial divisions. However, in some androcytes of about the same age as those shown in Fig. 2 (e.g. Figs. 1 and 4), bodies appear whose nucleolar nature is evidenced by their usually rounded shape and their affinity for safranin. Later (Figs. 13, 14, &c.), a single fairly large nucleole is always present in the androcyte nucleus. But this nucleole, though sometimes, especially in the earlier stages (Fig. 4), rather irregular in shape, is usually more or less rounded; it is neither so irregular nor so large as the nucleolar masses of the preceding cell generations, which were found to consist of a nucleole about which more or less chromatin was aggregated.

The blepharoplast, originally (Fig. 1) very small, early begins to increase in size. In the androcytes shown in Fig. 2, each blepharoplast is somewhat elongated. The blepharoplasts of the cells shown in Figs. 3 and 4 (which are in the same antheridium as are those shown in Fig. 2) seem to have grown more or less in all dimensions, although that of Fig. 3 is longest in a direction oblique to the plane of the section. Whatever be the differences in this respect at this early period, the growth tendency very soon shows itself exclusively in an elongation (Figs. 5, 6, 7, &c.). Excepting that it lies in the cytoplasm, I cannot find that at this time there is any rule governing the position of the blepharoplast. It may lie in contact with the nucleus or with the plasma membrane, or it may be at any distance, or its different parts may be at varying distances, from the nucleus on the one side and from the plasma membrane on the other. In the early stages of its elongation (Figs. 5, 10, 12), the blepharoplast is likely to be more or less uneven in thickness; sometimes (Fig. 5) one end is noticeably thicker than the other. But as its growth goes on (Figs. 11, 13), it becomes a curved rod of quite

uniform thickness, excepting that it is slenderer toward the ends, one or both of which may be pointed. The blepharoplast is stained deep black in iron-alum-haematoxylin, and deep blue in the triple stain. Some authors—for example, Ikeno (1903) in his study of *Marchantia*, Arens (1907) and the van Leeuwen-Reijnvaans (1908) in their investigations of *Polytrichum*—have described the blepharoplast as growing only into a relatively short body to whose posterior end is attached another thread-like or band-like structure formed by a gradual differentiation within the cytoplasm. Something similar is reported by Walker (1913) for *Polytrichum*. I cannot confirm these descriptions. All the evidence furnished by my preparations indicates that the long, darkly stained rod (shown for example in Figs. 11–14) is a homogeneous body formed by the growth of the originally small blepharoplast. In this respect my conclusions agree with those reached by Yamanoichi (1908) and Miss Allen (1911) in studying the spermatogenesis of Ferns, by Woodburn (1911, 1913) and Miss Black (1913) working with various Hepaticae, and by Woodburn (1915) in his study of *Mnium*.

While the blepharoplast is still comparatively short, another definite cytoplasmic body appears (Figs. 5, 9, 10). This is the large *limosphere*, to use a term suggested by M. Wilson (1911). This body seems to correspond to the one which, in the androcytes of *Marchantia*, was called by Ikeno (1903) the '*chromatoider Nebenkörper*'. It is certain that in *Polytrichum*, at any rate, the body in question is quite dissimilar in behaviour and history to the structure appearing in the spermatids of certain animals to which the name *chromatoider Nebenkörper* was first applied; and for this reason the term introduced by Wilson will be used in the present paper. The limosphere is at first rather faintly stained (commonly taking the orange of the triple stain); it seems to arise by a concentration of a portion of the cytoplasm. Indeed, the bodies shown in Figs. 9 and 10 are so slightly differentiated from the rest of the cytoplasm by their tint and their greater degree of homogeneity that one can hardly be certain either of their exact outline or of their identity with the body which shortly afterwards is so conspicuous. However, a comparison of these figures with Figs. 5 and 12, in which the limosphere is very distinct, although orange in colour, and with Fig. 13, in whose cells also the limosphere is slightly stained, leaves little doubt that it is the same body which is seen in each case. Except for a brief period at the beginning of its existence, the limosphere is deeply stained by iron-alum-haematoxylin and by the safranin of the triple stain. As a rule, the outer portion of the limosphere stains more deeply than most of its internal substance, so that it looks much like a hollow sphere (or, as Figs. 11, 12, &c., show, a ring in cross section). Very commonly there is also some deeply-stained material in the interior (Figs. 11, 12); but sometimes (Fig. 21) the stain is confined entirely to the outer shell. At still other times the limosphere seems to be uniformly stained throughout. This

is especially likely to be true shortly after its first appearance (Figs. 9, 10, 13); it is possible that in such cases there is no differentiation as yet between its outer and inner portions, although sometimes (e.g. Figs. 5 and 12) this differentiation certainly appears at a very early stage. The sections from which Figs. 14, 16, 17, 18, and 20 were drawn were stained first in gentian violet and then in safranin, thus reversing the usual order. Under this treatment the limosphere becomes deep red throughout; probably its apparent homogeneity in these cases is due merely to the unusually deep colour and consequent opacity of its outer shell. This notion is supported by the differentiation shown in the limosphere of Fig. 15, which is drawn from the same antheridium as are Figs. 14 and 16.

While the blepharoplast is growing, the arrangement of nucleus, blepharoplast, and limosphere seems decidedly variable. Cells may be found (compare those represented in Figs. 9–13) which show these three bodies in almost every conceivable spatial relation to one another as well as in almost every possible position within the cell. It does not follow, of course, that there is no relation between the positions of the nucleus, blepharoplast, and limosphere because no constancy in this respect is to be detected. When the androcyte is first formed, it plainly has a polar organization. Later, as we shall see, the different cell organs take up very definite positions with reference to one another, and the cell again displays a marked polarity. It is not necessary to assume that the polarity manifested during the earlier and the later history of the cell is lost in the period now under consideration, simply because, in the changes and shiftings incident to the formation of the limosphere and the elongation of the blepharoplast, these bodies take various positions with reference to the nucleus and to one another.

Fig. 13 shows a condition that is not infrequently found—namely, the presence at the distal end of the antheridium of a small group of androcytes (represented by the two uppermost ones in this figure) that are much larger than any others in the same antheridium and are provided with proportionally large nuclei and blepharoplasts. Similarly placed groups of large androcytes have been found at somewhat later stages than that here shown, but it was impossible to distinguish the details of their structure and they appeared to be in process of breaking down; no unusually large antherozoids have been found; and it is not unlikely that in all cases the giant androcytes cease their development comparatively early and become disorganized. Possibly comparable in a measure with these are the ‘double spermatids’ of *Mnium* observed by Wilson (1911), which are of about double the normal size, each containing two blepharoplasts, two limospheres, and probably two nuclei. Ikeno (1903) also describes an unusual growth of occasional androcytes in *Marchantia*; however, he thinks these giant cells divide, each thus forming two androcytes of normal size.

By the time the growing blepharoplast has reached a length equal to from two-thirds to three-fourths of the circumference of the cell (Figs. 14–20) its position seems to be more definitely fixed. It now lies close to, and for the most part in contact with, the plasma membrane, with one end near, but apparently not yet touching, the limosphere. This end of the blepharoplast will be referred to hereafter as its anterior end because it is to be concerned in forming the anterior portion of the body of the antherozoid. Arens (1907) finds that in both *Polytrichum* and *Mnium* the anterior end (constituting the whole of what he calls the blepharoplast) lies usually at that side of the androcyte which is towards the apex of the antheridium. I have not found any such regularity in the position of the blepharoplast in my material, nor has Wilson (1911) in his study of the androcytes of *Mnium*. In Figs. 14, 15, 16, and 19, only that part of the blepharoplast is shown which lies on the side of the androcyte turned towards the observer; in Figs. 17 and 18 the blepharoplast is shown throughout most or all of its length. The cell represented in Fig. 20 is unusual in the fact that the blepharoplast is in contact throughout the greater part of its length with the nuclear membrane and so at some distance from the plasma membrane. Apparently it is at about this time that the cilia first appear. At any rate, the youngest cilia-bearing cells that I have seen are those shown in Figs. 16 and 19. In each case the single cilium that is visible seems to be attached to the blepharoplast somewhat behind the anterior end of the latter. It should be noted that the methods used in staining and dehydrating the sections containing these cells were such as to leave little or no colour in structures so slender as the cilia; and also that in sectioning the material the cilia of any particular androcyte are likely to have been in whole or in part cut away. These facts doubtless explain why cilia are often not visible, even in the cases of cells much older than those shown in Figs. 16 and 19; and it is quite possible that the cilia begin their growth at a period earlier than that at which I have first seen them.

In the stages now under discussion, a dark-staining cytoplasmic granule, much smaller than the limosphere, is often conspicuous. Sometimes this granule seems to lie in a vacuole (Figs. 19, 25); sometimes it is elongated (Fig. 16); sometimes there are two similar granules (Fig. 18), of which one may be in a vacuole (Fig. 24, lowermost cell); sometimes (Figs. 14, 15) no such definite body is to be seen. Thus appearances are too variable at this time to justify any general statement concerning these bodies; but it is not improbable that one or more of them may correspond to a similar though larger body which at a somewhat later period seems to be a nearly or quite constant constituent of the androcyte.

The nucleus, which as a rule has remained thus far in the central region of the cell, now moves towards the periphery on that side at which the middle portion of the blepharoplast lies (Fig. 21). The nucleus becomes

pressed against the blepharoplast and the plasma membrane in this region. Fig. 19 represents a cell whose nucleus has assumed the peripheral position fairly early; in other respects, however, this cell seems to be in about the same stage of development as are those shown in Figs. 17 and 18, whose nuclei are still centrally located.

The next conspicuous change to be noted in the developing androcyte is a division of the limosphere. The division is preceded by an elongation of the limosphere in a direction perpendicular to the anterior portion of the blepharoplast, to which the limosphere now seems, at least in most cases, to be attached (Figs. 21, 22). Then follows a constriction (Figs. 23, 24), and finally a division (Figs. 25, 26) into two bodies which usually differ noticeably in size. The smaller of the two, hereafter referred to as the *apical body*, remains in contact with the anterior end of the blepharoplast; the larger one, since it is of approximately the size, and retains essentially the appearance, of the limosphere before its division, will still be spoken of as the *limosphere*. It moves away from the anterior end of the blepharoplast (Fig. 26) and soon comes to lie in contact with the nucleus (Pl. XVI, Fig. 27). The apparently divided limosphere shown in Fig. 20 (Pl. XV) represents a different condition; the androcyte from which this drawing was made is the only one I have found at so early a stage which contained a double (or constricted) limosphere, although less marked irregularities in the outline of this body have sometimes been observed. It is possible, though I think hardly likely, that the condition in Fig. 20 indicates a premature division of the limosphere. Something apparently equally removed from the usual course of events is shown in the lowermost androcyte of Fig. 24, whose limosphere seems to be dividing into four bodies instead of two.

Sometimes, when the apical body and the limosphere are moving away from each other (Fig. 28, Pl. XVI), they seem to be connected by a definite fibre or strand. This has not been observed in the majority of cases, but in many of the androcytes in the antheridium from which Fig. 28 was drawn this connecting strand is plainly visible. If it is generally present at these stages, the staining methods used are not adequate invariably to demonstrate the fact.

At about the time that the limosphere divides, the nucleus begins to elongate in a direction parallel to the length of the blepharoplast. The earliest evidences that I have found of the extension of the nucleus are in the androcytes shown in Figs. 22 and 23, Pl. XV. In each of these cases the nucleus is drawn out into a point that is directed towards the anterior end of the blepharoplast, the posterior part of the nucleus remaining rounded. The elongation of the nucleus seems usually to begin in this way; but there are exceptions to this rule, as in the androcyte shown in Fig. 25, whose nucleus is rounded anteriorly and somewhat drawn out at its

posterior end. It is evident from my preparations that the nucleus does not always begin to elongate at precisely the same stage; for the nuclei of Figs. 26 (Pl. XV) and 27 (Pl. XVI) show no evidence of a change in shape at a time when the apical body has been completely separated from the limosphere.

The further extension of the nucleus is the central feature of the later history of the androcyte. In this process the nucleus stretches out along, and becomes closely applied to, the blepharoplast. Usually the anterior beak of the nucleus increases in length (Fig. 30), the posterior end remaining rounded, sometimes to a relatively late period (Fig. 35). Sooner or later, however, the posterior end also becomes pointed. This may occur quite early (Figs. 28, 31), and probably always takes place before the nucleus has attained a length anywhere nearly equal to the circumference of the cell (compare Figs. 36, 38, and 39). As a rule the anterior portion of the nucleus continues to stretch more rapidly than the posterior portion, consequently the latter remains much thicker down to a very late stage (Figs. 39-41, 43, 44). Fig. 42 represents a condition rarely found, in which the posterior part of the nucleus has developed a slender prolongation nearly as long as that derived from the anterior part; as a result, the nucleus in this instance is thickest in its middle portion. Fig. 47 shows a somewhat similar condition at a later period, in which, however, the posterior prolongation is much shorter than the anterior one. The anterior prolongation of the nucleus is sometimes (Figs. 42, 43) bent into a double curve, as though an external resistance had been encountered in the course of its extension.

Apparently the anterior end of the nucleus never extends quite to the anterior end of the blepharoplast (Figs. 35-38). As the elongation of the nucleus continues and its anterior part becomes more slender until its diameter is little greater than that of the blepharoplast (Figs. 39-44), it becomes increasingly difficult to determine exactly how far forward the nucleus extends. However, even at these later stages, the anterior end of the continuous body that is now being formed by the blepharoplast and nucleus is stained exactly as the blepharoplast was stained before—being, for example, coloured somewhat more deeply by iron-alum-haematoxylin than the chromatic materials of the nucleus, and sometimes retaining the stain when the substances that are certainly nuclear are quite decolorized; it seems highly probable that this, which is to be the anterior extremity of the body of the antherozoid, is formed wholly from the substance of the blepharoplast. This conclusion is supported by the visible persistence of the apical body to a time when the body of the antherozoid is approaching maturity (Figs. 48, 49). The apical body, as we have seen, lies in contact with, and often flattened against, the end of the blepharoplast (Figs. 40, 41, 44, 48, 49). If the prolongation of the nucleus were to extend to the tip

of the blepharoplast, it must push in between the blepharoplast and the apical body; but of such an intrusion there is no indication.

Whether the nucleus, as a result of its elongation, finally extends to or beyond the posterior end of the blepharoplast, I have not been able in most cases to determine. As will appear more fully later, the blepharoplast, except for its short anterior portion, usually becomes indistinguishable while the nucleus is still in the early stages of extension. Rarely (as in Figs. 45 and 46) the blepharoplast is visible as a distinct body until a quite late period. In Fig. 45, representing an androcyte, the posterior portion of whose nucleus is towards the observer, the anterior end of the blepharoplast is seen close to the apical body in the upper end of the figure; it can be traced in the preparation from which the drawing was made, along the anterior half of the nucleus (not shown in the figure), and then along the posterior half of the nucleus, which, with the corresponding part of the blepharoplast, appears in the drawing. The posterior tip of the nucleus, and perhaps also that of the blepharoplast, are hidden from view by the limosphere. Fig. 46 shows a different view of an androcyte found in the same antheridium. In this case also the blepharoplast can be traced nearly the full length of the nucleus. The anterior portion of the blepharoplast, apparently extending beyond the end of the nucleus, is shown, and what seems to be the posterior end of the blepharoplast appears near the upper edge of the figure, in close contact with the nuclear membrane. Here it appears that, just as the blepharoplast extends beyond the nucleus anteriorly, posteriorly the nucleus extends beyond the blepharoplast.

The nucleole usually remains conspicuous until the later stages of nuclear elongation (Figs. 43, 45). Thus far there has been no decrease in the bulk of the nucleus; rather, to judge from appearances, the opposite has happened, for, as the figures show, the nuclei in process of elongation often seem much larger than those of young androcytes (compare, e. g., Figs. 31, 37, and 38, Pl. XVI, with Figs. 14-20, Pl. XV). Some of the apparent increase in size is due to the fact that as the nucleus is elongating it also flattens itself against the plasma membrane, thus, in certain views, giving an exaggerated impression of its bulk. Allowing for this source of error, however, it seems true that the progressive change in shape of the nucleus thus far described is actually accompanied by an increase in its size.

When the nucleus has reached nearly or quite its final length (Figs. 43, 44, 47, Pl. XVI), a portion of it still remains comparatively thick. Now ensues a period (Figs. 45, 46, 48-55) during which the diameter of the nucleus (and consequently its volume) is considerably diminished, not only in the region that has thus far remained thick but in its slenderer portions as well. Until this time the reticulate structure of the nuclear contents has persisted. The diminution in volume which now occurs seems to involve the extrusion of the nuclear sap. As a result, the

chromatic constituents (including perhaps the nucleole, which is no longer recognizable) are pressed more closely together (Figs. 48, 49), and finally (Figs. 50-55) the body of the antherozoid, consisting of the nucleus intimately united with, and indistinguishable from, what remains of the blepharoplast, becomes a slender, coiled chromatic rod, somewhat slenderer or even pointed at its anterior and posterior ends. The exact degree of coiling of this body, and perhaps also its length, vary in different antherozoids (compare, e. g. Figs. 51 and 52); as a rule, the body constitutes about one and one-half coils of a spiral.

While the nucleus is undergoing the changes just described, the body of the blepharoplast, except for the short portion at its anterior end, becomes indistinguishable. This is sometimes the case even in the very early stages of nuclear elongation (Figs. 25, Pl. XV; 27, Pl. XVI). However, in other cells at corresponding stages (Figs. 26, Pl. XV; 30 and 31, Pl. XVI), and even sometimes at a considerably later period (Figs. 32, 33, 35), the blepharoplast is still visible throughout its length. Indeed, in the cells shown in Figs. 32 and 35, the blepharoplast seems to be longer than it was seen to be at any earlier period, as though it had continued to grow in length after the change in shape of the nucleus had begun. Sometimes (Figs. 23, Pl. XV; 29, Pl. XVI) the blepharoplast can be followed for some distance backward from the point at which the anterior end of the nucleus is applied to it, but it disappears before the posterior portion of the nucleus is reached. It seems probable that the difficulty in distinguishing the greater part of the body of the blepharoplast is due, not to an actual disappearance of part of its substance, but to some change which makes it less conspicuous. This might be a change merely in its staining reactions; it might be in part due to a stretching of the blepharoplast after the nucleus has begun to elongate, with a resultant decrease in thickness; or it might be because of an intimate union with, and flattening along, the nuclear membrane. The last explanation, however, would not account for the invisibility of the posterior part of the blepharoplast, with which, at such stages as those of Figs. 23 and 25 (Pl. XV), no part of the nucleus is yet in contact; and, on the whole, a change in the reactions of the blepharoplast to stains seems the most probable explanation of its apparent disappearance. This notion is strengthened by such cases as those shown in Figs. 28 and 29, Pl. XVI. In the androcyte represented in Fig. 28, the part of the blepharoplast that is in contact with the nucleus cannot be traced with any certainty; but that part which extends posteriorly beyond the nucleus is visible, though faintly stained. In other androcytes in the same antheridium, the posterior portion of the blepharoplast is more conspicuous. Fig. 29 represents an androcyte found in another section of the same antheridium. In this cell the blepharoplast is plainly visible from its anterior end to about the middle of that part which touches the

nucleus; at the latter point the blepharoplast seems to end, although it is highly probable that conditions are really substantially alike in the two androcytes of Figs. 28 and 29. Sometimes the blepharoplast can be followed for nearly or quite its full length at a much later period, as in Figs. 45 and 46 already referred to. So it seems necessary to conclude, in spite of the frequent impossibility of tracing the blepharoplast throughout its length, that it actually persists without material decrease in size until a comparatively late stage in the history of the metamorphosis of the androcyte. Eventually, however, the blepharoplast (except for its anterior end) seems completely to disappear; and whether this final disappearance is due to its coalescence with the nuclear membrane or to an actual absorption or destruction of its substance, must be left unsettled.

In the later stages of nuclear elongation (Figs. 38-44), the anterior end of the blepharoplast seems to lie outside the boundary of the remaining cytoplasm—whether or not still covered by the plasma membrane is not clear. Occasionally, as in Fig. 44, the apical body as well as the end of the blepharoplast seems to be partly outside the cytoplasm. The projecting tip of the blepharoplast probably corresponds to the cytoplasmic *Höcker* described by Strasburger (1892), and also to the refractive *bouton* of Guignard (1889), although the latter author takes the swelling in question for the anterior end of the nucleus. The appearances in my preparations at this time suggest that the anterior end of the developing antherozoid is pushing out and thus beginning to free itself from the cytoplasm that is not to be used in its formation. Something like this is described by Ikeno (1903) as occurring in the spermatogenesis of *Marchantia*. But I do not find in *Polytrichum* a continuation of this process such as Ikeno's figures suggest for *Marchantia*, namely, a further pushing outward of the anterior end so that the unused cytoplasm is as it were forced to the posterior end of the antherozoid. On the contrary, even after the cytoplasm has greatly diminished in amount (Figs. 51, 52), a portion of what is left remains attached to the anterior end of the body of the antherozoid; and the final disappearance of this anterior mass of cytoplasm is one of the last events in the history of the androcyte.

The same difficulty in the observation of cilia that was noted earlier is met with throughout this history, although portions of one or both cilia are frequently to be seen (Figs. 29, 34, 42, 43, &c.). In sections containing mature or nearly mature antherozoids (Figs. 50-2, 54), considerable portions of the cilia are visible; but they can be seen in their full length and in their relation to the body only in preparations that contain unsectioned antherozoids (Figs. 56-9).

The apical body remains for a long time in contact with the anterior end of the blepharoplast. No apparent material change takes place in its size, form, or density down to the latest period (Figs. 48, 49) at which I have

observed it. It is sometimes approximately spherical (Figs. 25, Pl. XV; 34, 45, Pl. XVI); occasionally (Figs. 26, Pl. XV; 37, Pl. XVI) it is slightly elongated in an axis perpendicular to the blepharoplast; oftener it is more or less flattened against the blepharoplast (Figs. 28, 32, 40, &c.). Sometimes differences appear in the staining reactions of different parts of the apical body (Figs. 26, Pl. XV; 37, 38, 42, Pl. XVI); but there is no uniformity in these appearances, and as a rule the whole body is homogeneously stained. The latest stage at which I have seen the apical body is that shown in Figs. 48 and 49, at which the nucleus has attained approximately its final length. After this time the apical body seems to disappear very quickly and completely. Occasionally a mature antherozoid seems to have a slightly thicker portion just behind its pointed anterior tip (Fig. 59), which at times I have thought might be due to the retention of part or all of the substance of the apical body; although it may also be explained by the presence of the anterior portions of the cilia, lying close to, and indistinguishable from, the body of the antherozoid; at any rate, the part (if any) taken by the apical body in the formation of the mature antherozoid is so small as usually (Figs. 56–8) to produce no perceptible effect.

The limosphere, after the formation of the apical body, places itself, as already noted, in contact with the nucleus (Fig. 27). Sometimes (Fig. 28) the limosphere is flattened in the region of contact; oftener (Figs. 32, 35, 39) the nuclear outline is indented as though it had yielded where the limosphere pressed against it. When the nucleus begins to elongate, the limosphere is usually in contact with that portion of the nucleus which is least stretched out, and whose diameter therefore remains greatest (Figs. 28, 30, 35); and since the elongation of the nucleus is most marked at first in its anterior region, the limosphere thus lies nearer the posterior than the anterior end of the nucleus. Sometimes the limosphere lies at the very posterior end (Figs. 33, 37, 45), or close to this end (Figs. 36, 39, 40), of the nucleus, and therefore farther back than the region of greatest nuclear diameter. However, in the rare cases observed in which there is both a posterior and an anterior elongation of the nucleus (Figs. 42, 47), the limosphere is in contact with the thickest portion of the nucleus, and therefore at some distance from its posterior as well as from its anterior end. At the late stage shown in Figs. 48 and 49 the limosphere is still present, enclosed within the posterior part of the coiled nuclear body (Fig. 48) or at the very tip of the latter (Fig. 49). The same is true at still later stages (Figs. 50–3), and the limosphere is similarly placed in antherozoids seen in sections of nearly or quite mature antheridia (Figs. 54, 55). In sections showing these very last stages, there is no longer a visible differentiation between the outer and inner portions of the limosphere.

During that period in the metamorphosis of the androcyte which is represented by Figs. 30–5, a darkly staining body, distinct from both

the apical body and the limosphere, is generally if not invariably present. This body—hereafter referred to, because of its staining properties, as the *percnosome*—is variously located; thus, in Fig. 33 it is shown near the apical body; in Fig. 35, close to the limosphere and in contact with the posterior part of the nucleus; in Fig. 32, between the limosphere and the posterior part of the blepharoplast; in Fig. 31 (and perhaps in Fig. 30, where the percnosome is partly hidden by the nucleus) it is close to the posterior end of the blepharoplast. In the androcyte shown in Fig. 34 the percnosome was not seen, although it appeared in other cells in the same antheridium. The dark object lying over the apical body in Fig. 27, although comparatively small, is probably also the percnosome. As already noted, at still earlier stages in the history of the androcyte a single small cytoplasmic body is often present, sometimes lying in a vacuole. At later stages, also, there is commonly a single small, darkly stained granule located somewhere in the cytoplasm (Figs. 38, 39, 42), towards the close of the history usually in a vacuole (Fig. 47), and apparently becoming smaller (Figs. 48–50) as the development of the antherozoid proceeds. Occasionally at these, as at earlier stages, there is more than one darkly stained cytoplasmic granule (Figs. 41, 44, 46); but in such a case one of the bodies is usually darker and more definite in outline than the others. If these various appearances are properly to be interpreted as evidencing the persistence of the percnosome during the greater part of the history of the androcyte, it is evident that this body is very small at its first appearance, later grows considerably, and then in turn diminishes in size.

In Fig. 35 still another rounded body appears in the cytoplasm near the posterior end of the blepharoplast, and not far from the apical body, though at a somewhat different depth. In the cell from which this figure was drawn, the body in question was stained orange, as was also the apical body, while the percnosome was dark red, as is usual in triple-stained preparations. The orange-stained body in question was not seen in any other androcyte. Possibly it is to be classed among the disintegration products of those parts of the cytoplasm that are not used in the construction of the antherozoid; indeed, the same suggestion might be made regarding the percnosome but for the fact that its apparent long persistence seems to indicate for it some more special significance.

As the development of the body of the antherozoid proceeds, the volume of the androcyte cytoplasm gradually diminishes. The cell as a whole becomes more and more flattened or lenticular, its short axis being perpendicular to the plane in which the coiled body of the antherozoid approximately lies. This change of form is apparent in androcytes which, like those shown in Figs. 43 and 45, are so placed as to show the coiled nucleus more nearly in edge than in side view. Fig. 50 shows two anthero-

zoids in section at a much later stage, when what remains of the androcyte cytoplasm has been reduced to a very thin layer.

It is not easy to determine in just what order the cytoplasm in different parts of the androcyte disappears. It is possible that the vacuole so commonly present in the older androcytes (Figs. 46, 47), which seems to be somewhat larger in still older cells (Figs. 48-50), and which contains the granule that I have identified tentatively with the percnosome, indicates the beginning of the final disappearance of the unused cytoplasm, and that succeeding stages in the process consist in the progressive growth of this vacuole. At the very late stage shown in Figs. 51 and 52, the diminishing cytoplasmic substance is represented by two masses—one, the larger, is attached to the anterior portion of the antherozoid; a second, irregular in form and including the limosphere, is enclosed within the arc formed by the posterior end of the antherozoid. This latter cytoplasmic mass is best shown in Fig. 53, which represents the posterior part of an antherozoid in the same antheridium as those shown in Figs. 51 and 52. By the time of the maturity of the antherozoid (Figs. 54, 55), the anterior cytoplasmic mass has disappeared, but the posterior mass with the limosphere still remains. This persistent remnant of the unused cytoplasm is the cytoplasmic 'vesicle' of many authors.

The gradual swelling and softening of the walls separating the androcytes, which has been described as beginning very early—even before the division of the androcyte mother-cells—continue until the walls, as definite structures, entirely disappear. The androcytes now lie embedded in a substance, evidently derived from the disintegrated walls, which stains very faintly, often so faintly that it shows no stain at all except by contrast with the adjacent uncoloured portions of the preparation. After the walls are disorganized, each androcyte in an antheridium sometimes seems to be enclosed in an envelope (Figs. 41, 45, 50) composed of a material which is itself faintly stained but is distinct from, and evidently denser than, the substance which otherwise fills the spaces between the androcytes. Sometimes this special envelope is only faintly visible, and often it is quite indistinguishable. Whether or not it is always present but visible only in specially favourable cases must be left an open question. When present, the envelope conforms in general to the shape of the androcyte, being therefore lenticular in the later stages (Fig. 50); but it is turgid, and the space within it is by no means completely filled by the androcyte.

In antheridia containing mature antherozoids (Figs. 54, 55) I have never been able to distinguish this envelope. Antheridia at this stage do, however, differ markedly with reference to the stainability of the substance in which the antherozoids are embedded. In the antheridium from which Fig. 55 was drawn, each coiled antherozoid lies in a clear space

or vesicle, whose form is that of the space enclosed in one of the definite envelopes of a somewhat earlier period (Fig. 50); between these vesicles is an orange-stained substance of variable density. The antheridium from which Fig. 54 was made shows no such distinction between vesicular and intervesicular materials; and the substance in which the antherozoids lie shows no stain, excepting that at places where it abuts upon a perfectly clear space it is seen by contrast to have a faint tinge. These two very different conditions may be seen in antheridia lying side by side in the same section, so that it can hardly be due to differences in the exposure to stains; Figs. 54 and 55 were drawn from different sections (in the same preparation) of antheridia from the same head. It is possible that these differences indicate different stages in the development of the antheridium; but I find nothing to support this notion, and consider it more likely that they represent variations in the conditions within different antheridia.

As the antheridium approaches maturity, there is a considerable shrinkage in the mass of its contents, including the androcytes (or antherozoids) and the substances in which they are embedded. This shrinkage is observable both in fixed and in living antheridia; the contents are contracted, usually towards one side, leaving a large part of the cavity of the antheridium apparently empty. This contraction is doubtless due largely or entirely to the fact that the mature antherozoid is much smaller than the androcyte from which it has been formed, most of the cytoplasm of the latter having disappeared and the antherozoid having taken the form of a flattened coil.

When the opening of a mature antheridium in a drop of water is observed under the microscope, each antherozoid is seen to be enclosed in a sharply outlined drop of clear liquid which is embedded in turn in a denser substance. It is this appearance that has led various observers to describe the antherozoid as enclosed in the wall, or the remains of the wall, of the 'mother-cell'. That there is nothing of the nature of a wall is plain from the history already outlined, as well as from the fact that the substances surrounding the antherozoids gradually dissolve in water. For some time the antherozoids can be observed in rapid movement, each within its own vesicle. As the intervesicular substance is gradually dissolved, the vesicles become separated from one another; but the tenacity of the membrane or layer immediately surrounding each vesicle is such that, even though the mass escaping from an antheridium be left in water for as long as two hours, only an occasional antherozoid escapes from its vesicle. Figs. 56-9 represent antherozoids which were fixed and stained after being set free from the antheridium. Those shown in Figs. 56 and 57 were still enclosed in their vesicles when fixed. It is noteworthy that the outline of the vesicle is quite invisible in such a preparation, and that the intervesicular substance

is represented, if at all, only by a faint tinge, although it was plainly visible before fixation. Doubtless the substances surrounding and separating the antherozoids were largely dissolved in the liquids through which the preparation was passed. The antherozoid shown in Fig. 58 is beginning to free itself, probably having pushed its anterior end beyond the boundary of its enclosing vesicle; and that in Fig. 59 is quite free. The difficulty that the antherozoids of *Polytrichum* apparently find in freeing themselves from the enclosing vesicles has been noted by other observers (most recently by Walker, 1913), in contrast with the readiness with which the antherozoids of other Mosses are set at liberty. It may well be that the conditions in this respect are different in nature from those which obtain in a drop of water under observation in the laboratory.

Each antherozoid in motion shows under a comparatively low power of the microscope a refractive spot; with the oil-immersion objective this spot is seen to be a hollow sphere containing many granules in Brownian movement. On one occasion, when an antherozoid that had become free was under observation, this sphere was seen to burst. There can be no question, I think, that the sphere in question is the limosphere, still embedded in the cytoplasmic mass that adheres to the posterior end of the antherozoid. In the case of fixed and stained antherozoids (Figs. 56-9), the cytoplasmic remnants include various darkly staining bodies, among which it is not usually easy to distinguish the limosphere. Apparently, in the course of the processes concerned in making the preparations, the limosphere is often burst. Attached to the posterior tip of the antherozoid shown in Fig. 57 is a body of somewhat irregular shape whose outer portion is darkly stained; this is probably the limosphere, but the identification is not certain. I have not observed the final discarding of the cytoplasmic remnants by the moving antherozoid; but there can be little doubt that, as in the case of the antherozoids of other bryophytes, this now useless material is thrown off at some time before fertilization occurs.

The cilia are apparently attached to the body of the antherozoid a short distance behind its anterior end. Some of the published figures of Moss antherozoids show the cilia diverging from the extreme tip of the body. It is possible in any individual case that the cilia remain appressed against the body, and therefore indistinguishable from it, for a greater or less distance behind their real point of attachment; but this would hardly happen in every instance, and in a careful study of hundreds of antherozoids I have not found one which gave an indication of an attachment of the cilia otherwise than as shown in Figs. 56-9.

From the description which has now been given of the processes concerned in spermatogenesis, it appears that the material of which the body of the antherozoid is composed has been derived, so far as our present methods allow us to determine, entirely from the blepharoplast and the

nucleus. It is possible that some of the substance of the apical body is retained at the tip of the antherozoid; but of this there is no direct evidence. Except for this possibility, a short portion at the anterior end seems to have been formed by the blepharoplast alone. The remainder of the body consists chiefly of the metamorphosed nucleus; and, if the greater part of the blepharoplast persists at all in the mature antherozoid, it has become so closely appressed against, or united with, the nuclear membrane as to be indistinguishable. A certain difference in reaction to stains is observable between the anterior end and the remainder of the body of the antherozoid. For example, if an antherozoid be stained with safranin and pyoktanin blue, its anterior part shows a greater affinity for the safranin, in which respect it resembles the cilia; the rest of the body takes more of the blue stain. But there is no sharp line of demarcation; rather, the reddish stain at the tip shades gradually into a purple and this in turn into a blue.

The cilia are undoubtedly formed of cytoplasmic substance, probably similar in nature to that of the blepharoplast. The limosphere and percnosome play no recognizable part in the development of the antherozoid, although the latter probably persists until a very late period and the former body is still present in the cytoplasmic remnants that are attached to the mature antherozoid.

THE STRUCTURES OF THE ANDROCYTE IN VARIOUS PLANTS.

Leaving out of consideration the conflicting views concerning the origin and homologies of the blepharoplast, which have been discussed in another place (Allen, 1912), the results of recent work are in substantial accord as to the general features of the history of the blepharoplast and nucleus in the androcytes of those plants whose spermatogenesis has been studied. With reference to the Liverworts, Mosses, and true Ferns, perhaps the most marked difference of opinion that still exists is over the question whether the long cord or band to which the elongating nucleus becomes applied is developed from the blepharoplast alone, or partly from the blepharoplast and partly from a later-formed structure. Upon this point most observers hold to the former view—a view with which the present writer agrees. In *Equisetum* and *Marsilea* (Sharp, 1912, 1914), as in the Cycadales, the blepharoplast, instead of simply growing longer, breaks up into a mass of granules which later unite to form a continuous thread. While the possibility of a somewhat similar occurrence is suggested by the rather knotty appearance of the blepharoplast of *Polytrichum* when it begins to elongate, there is no time when it is visibly resolved into smaller bodies. The spermatogenesis of the Cycadales and *Ginkgo* is distinguished by the fact that the nucleus does not elongate; at most it pushes out a short beak towards the blepharoplast. In these Gymnosperms, too, in contrast with the conditions in Bryophytes and Pteridophytes, all the cytoplasm of the androcyte seems to remain as an

integral part of the antherozoid. As between the Characeae and all the higher groups, an important difference seems to obtain in the fact that in the former, according to the most recent account (Mottier, 1904), the blepharoplast appears first as a slender thread instead of originating as an isodiametric body which later elongates.

With reference to the other structures of the androcyte, the accounts of different observers are extremely confusing. It is highly probable, as already pointed out, that the '*chromatoider Nebenkörper*' described by Ikeno (1903) in the androcyte of *Marchantia* corresponds to the limosphere of *Polytrichum*. As Ikeno points out, Schottländer (1892) had figured a spherical body in the androcyte of *Marchantia* which is probably the '*Nebenkörper*'. Bolleter's (1905) and Humphrey's (1906) descriptions of similar bodies in the androcytes of *Fegatella* and *Fossombronia* are in harmony with that of Ikeno. Humphrey describes the body in question in *Fossombronia* as taking a position between the blepharoplast and the nucleus, where it elongates somewhat—a behaviour that is suggested by one of Ikeno's figures. Finally, Humphrey thinks, the '*Nebenkörper*' becomes a part (the 'middle piece') of the body of the antherozoid.

Arens (1907) finds, in the androcytes of *Mnium*, a body which he homologizes with the '*Nebenkörper*' of *Marchantia* and *Fossombronia*. The body seen by Arens seems unquestionably to be the same as the limosphere of *Polytrichum*. According to him, it moves to a position between the nucleus and the blepharoplast and is there dissolved, its former position being marked in the later stages of spermatogenesis by a vacuole. If it be remembered that what Arens calls the blepharoplast is but the anterior end of the long body to which the majority of authors have applied that name, it will be seen that the behaviour of the '*Nebenkörper*' in *Mnium* (as well as that of the similarly named body in *Marchantia* and *Fossombronia*) corresponds closely with the behaviour of the limosphere as I have followed it in *Polytrichum* down to the time at which it gives off the apical body.

The van Leeuwen-Reijnvaans (1908) describe a corresponding body, also under the name '*chromatoider Nebenkörper*', in the androcytes of *Polytrichum*. Their figures show that they observed it also taking a position close to the anterior end of the blepharoplast, where they think it disappears before the nucleus begins to elongate.

The bodies appearing in the androcytes of *Polytrichum*, *Atrichum*, and *Pellia* to which Wilson (1911) gives the name *limosphere*, are also in general certainly to be identified with that for which I have used the same name. But Wilson's description is so confused, and so many of the appearances he describes are evidently the result of faulty technique, that it is sometimes difficult to determine whether at different stages he is applying the same name to the same structure.

Walker's (1913) figures of the developing androcytes of *Polytrichum formosum* come much nearer to giving a complete history of the limosphere than do those of any previous writer. In his description, unfortunately, Walker has confused the anterior and posterior ends of the blepharoplast and of the nucleus, and has not altogether succeeded in distinguishing artefact from normality.

Woodburn (1915) shows the limosphere plainly in several of his figures of stages in the spermatogenesis of *Mnium*. He seems, however, to be quite uncertain as to the permanence, or even the identity in different cases, of the body that he figures.

The bean-shaped 'amylaceous mass', described by Guignard (1889) in the androcyte of *Sphagnum*, which persists in the cytoplasmic vesicle attached to the posterior end of the antherozoid, suggests in its behaviour an analogy at least to the limosphere of *Polytrichum*. Campbell (1887) had seen the same body in the vesicle of the antherozoid of *Sphagnum*, and had concluded from its reactions with iodine that it was starch. Still earlier, Roze (1864) had made a similar observation. It remains for present-day cytological methods to determine how closely *Sphagnum* agrees, in this feature of the history of its androcyte, with the Bryales that have been investigated. In this connexion it may be a pertinent fact that several writers have described starch grains as present in some numbers in the cytoplasmic vesicles of the antherozoids of Pteridophytes.

The '*Nebenkern*' found by Yamanouchi (1908) in the androcyte of *Nephrodium* might be thought to correspond either to the limosphere or to the percnosome. Its small size during most of the history and its variable position within the androcyte make its identification with the latter body seem more probable; but its larger size in the later stages of spermatogenesis and its persistence in the cytoplasmic vesicle that is attached to the posterior end of the mature antherozoid suggest a resemblance to the limosphere. It is possible that the very small *Nebenkern* of the early part of this history and the somewhat larger body observed in later stages are not, as Yamanouchi evidently believes, identical. There is even greater uncertainty as to the identity of a body described by Thom (1899) as a 'blepharoplast' or '*Nebenkern*' in the androcytes of *Aspidium* and *Adiantum*. The fact that he finds this body still present and unchanged in form after the elongation of the nucleus has begun seems to indicate that he is dealing, not with the blepharoplast as he supposes, but with another body which probably corresponds to Yamanouchi's '*Nebenkern*'.

The two large, spherical cytoplasmic bodies which Hirasé (1898) first observed in the body cell (androcyte mother-cell) of *Ginkgo* are also possibly homologous with the limosphere. In the division of the mother-cell, one of these bodies passes into each androcyte, where it persists throughout the development of the antherozoid, and is visible in the cytoplasm of the latter

until the moment of fertilization. Another conspicuous body, irregular in form, which appears in the cytoplasm of the mother-cell, passes into one of the two antherozoids and is described also as persisting to the time of fertilization. The latter is, perhaps, the only structure so far reported in plants which may be imagined to be in any way comparable with the sex chromosomes of animals—with the important difference that the resulting cytological differentiation between the male germ cells of *Ginkgo* is in no way connected with chromosome reduction. The case is of special interest because of the strict dioecism of *Ginkgo*.

The van Leeuwen-Reijnvaans (1908) describe a small chromatic body in the androcyte of *Polytrichum*, distinct from the blepharoplast and the limosphere, which at one stage lies in contact with the nucleus and also, to judge from their figure, with the posterior end of the blepharoplast. It is likely that this body is the percnosome. The 'accessory body' described by Wilson (1911) in the androcytes of *Polytrichum*, *Atrichum*, and *Pellia* is also probably in general to be identified with the percnosome.

Walker (1913) shows in several of his figures a small but conspicuous cytoplasmic body (in his Fig. 34 there are two such bodies) in the androcyte of *Polytrichum*. This body, which is without much doubt also the percnosome, is shown in several cases, notably in his Fig. 36, as lying at the posterior end of the blepharoplast. Walker seems to consider the body so located to be the blepharoplast itself—the long cord which is really the elongated blepharoplast being taken by him for a distinct structure.

Nothing shown in Woodburn's (1915) figures of the spermatogenesis of *Mnium* is recognizable with any degree of certainty as the percnosome. In one case (his Fig. 15) he shows a small body lying in a vacuole; in another (his Fig. 14) there is a similar appearance, but in this case he describes the body in question as probably lying upon the surface of the vacuole, and the 'vacuole' itself, which is partly surrounded by a darkly stained layer, is evidently the limosphere. Lewis (1906) and Miss Black (1913) likewise find a single large vacuole, but nothing resembling the percnosome, at certain stages in the history of the androcytes of *Riccia natans* and *R. Frostii*.

Apparently no previous writer has recognized the structure that I have called the *apical body*. However, Walker (1913) figures in certain cases what is probably this body; especially in his Fig. 33, which shows two structures side by side that are quite certainly the limosphere and the newly formed apical body, the latter being in contact with the anterior end of the blepharoplast.

SUMMARY.

Each newly formed androcyte of *Polytrichum juniperinum* contains a small rounded blepharoplast which behaves like a centrosome in the division of the androcyte mother-cell, and which in most cases still lies in the region recently occupied by a spindle pole.

The blepharoplast elongates, places itself in contact with the plasma membrane, and ultimately forms a long, peripherally placed, curved cord. Two long cilia grow out from it; their point of attachment is a short distance behind the anterior end of the blepharoplast.

The nucleus moves into contact with the blepharoplast and stretches out along the latter. The blepharoplast, though visible until a comparatively late stage, ultimately becomes indistinguishable from the nucleus except for its anterior end, which apparently projects a short distance beyond the tip of the elongated nucleus. The nucleus becomes a long, slender, coiled, finally homogeneous body, of about one and one-half turns. The nucleus and the blepharoplast seem to constitute the whole of the body of the mature antherozoid.

At about the time the blepharoplast begins to elongate, a large, spherical body, the limosphere, appears, variously situated in the cytoplasm; soon it comes to lie close to the anterior end of the blepharoplast. In this position it divides unequally; its smaller portion becomes the apical body, its larger retains the appearance of the limosphere before the division and is referred to by the same name.

The apical body remains applied to the anterior end of the blepharoplast until a very late period, but it has not been clearly shown to take any part in the formation of the body of the antherozoid.

The limosphere, after the formation of the apical body, takes a position in contact with the nucleus, nearly always with the posterior portion of the latter. The limosphere persists in the cytoplasm until the time of the maturity of the antherozoid.

During certain stages in the history of the androcyte, another conspicuous cytoplasmic body, the percnosome, seems to be regularly present. It is probably identical with a smaller granule which is generally recognizable both at an earlier and at a later period. This smaller body sometimes—at very late stages usually, if not always—lies in a rather large vacuole.

During this history the androcyte becomes approximately spherical, then, as the bulk of the cytoplasm decreases, lenticular. A portion of the cytoplasm, including the limosphere, remains included within the curve of the posterior end of the mature antherozoid.

The walls which originally separated the androcytes gradually soften and become dissolved. Each antherozoid, when mature, lies in a vesicle which, seen as the contents ooze out of the antheridium, seems to be bounded

by something like a distinct membrane; but in fixed material no trace of such a membrane is to be found at this time, although a similar structure does seem to be present in preparations of some antheridia at an earlier stage. The separate vesicles are embedded in a viscous substance which is probably derived from the material of the broken-down walls.

A part of the work upon which the present paper is based was carried on in the laboratory of Professor Mangin at the Muséum d'histoire naturelle, Paris. It is a pleasure to record here my appreciation of the unfailing courtesy of M. Mangin and his assistants, and of the generosity with which the facilities of the laboratory were placed at my disposal.

THE UNIVERSITY OF WISCONSIN.

LITERATURE CITED.

- ALLEN, C. E. (1908): The Cytology of the Male Gametophyte of *Polytrichum juniperinum*. Science, N. S., vol. xxvii, p. 334.
- (1912): Cell Structure, Growth, and Division in the Antheridia of *Polytrichum juniperinum*, Willd. Arch. f. Zellforsch., Bd. viii, p. 121.
- (1913): A Comparison of Plant and Animal Spermatogenesis. Science, N. S., vol. xxxvii, p. 383.
- (1914): Development of the Male Germ Cells of *Polytrichum*. Science, N. S., vol. xl, p. 775.
- ALLEN, RUTH F. (1911): Studies in Spermatogenesis and Apogamy in Ferns. Trans. Wisc. Acad. Sci., Arts, and Letters, vol. xvii, pt. i, p. 1.
- ARENS, P. (1907): Zur Spermatogenese der Laubmoose. Inaug. Diss., Bonn.
- BLACK, CAROLINE A. (1913): The Morphology of *Riccia Frostii*, Aust. Ann. of Bot., vol. xxvii, p. 511.
- BOLLETER, E. (1905): *Fegatella conica* (L.), Corda. Eine morphologisch-physiologische Monographie. Beih. z. Bot. Centralbl., Bd. xviii, Abt. 1, p. 327.
- CAMPBELL, D. H. (1887): Zur Entwicklungsgeschichte der Spermatozoiden. Ber. deut. bot. Ges., Bd. v, p. 120.
- GUIGNARD, L. (1889): Développement et constitution des anthérozoïdes. Rev. gén. de Bot., t. i, pp. 11, 63, 136, 175.
- HIRASÉ, S. (1898): Études sur la fécondation et l'embryogénie du *Ginkgo biloba* (Second mémoire). Jour. Coll. Sci. Tokyo, vol. xii, p. 103.
- HUMPHREY, H. B. (1906): The Development of *Fossombronina longiseta*, Aust. Ann. of Bot., vol. xx, p. 83.
- IKENO, S. (1903): Beiträge zur Kenntnis der pflanzlichen Spermatogenese; Die Spermatogenese von *Marchantia polymorpha*. Beih. z. Bot. Centralbl., Bd. xv, p. 65.
- VAN LEEUWEN-REIJNVAAN, W. and J. (1908): Über eine zweifache Reduktion bei der Bildung der Geschlechtszellen und darauf folgende Befruchtung mittels zwei Spermatozoiden und über die Individualität der Chromosomen bei einigen *Polytrichum*-Arten. Rec. trav. bot. néerl., t. iv, p. 177.
- LEWIS, C. E. (1906): The Embryology and Development of *Riccia lutescens* and *Riccia crystallina*. Bot. Gaz., vol. xli, p. 109.
- MOTTIER, D. M. (1904): The Development of the Spermatozoid in *Chara*. Ann. of Bot., vol. xviii, p. 245.

- ROZE, E. (1864): Recherches sur les anthérozoïdes des Cryptogames. Bull. Soc. bot. France, t. xi, pp. 225, 293.
- SCHOTTLÄNDER, P. (1892): Beiträge zur Kenntniss des Zellkerns und der Sexualzellen bei Kryptogamen. Cohns Beitr. z. Biol. der Pflanzen, Bd. vi, p. 267.
- SHARP, L. W. (1912): Spermatogenesis in *Equisetum*. Bot. Gaz., vol. liv, p. 89.
- (1914): Spermatogenesis in *Marsilia*. Bot. Gaz., vol. lviii, p. 419.
- STRASBURGER, E. (1892): Schwärmsporen, Gameten, pflanzliche Spermatozoiden und das Wesen der Befruchtung. Histologische Beiträge, iv, p. 47. Jena.
- THOM, C. (1899): The Process of Fertilization in *Aspidium* and *Adiantum*. Trans. Acad. Sci. St. Louis, vol. ix, p. 285.
- WALKER, N. (1913): On Abnormal Cell-fusion in the Archegonium; and on Spermatogenesis in *Polytrichum*. Ann. of Bot., vol. xxvii, p. 115.
- WILSON, M. (1911): Spermatogenesis in the Bryophyta. Ann. of Bot., vol. xxv, p. 415.
- WOODBURN, W. L. (1911): Spermatogenesis in certain Hepaticae. Ann. of Bot., vol. xxv, p. 299.
- (1913): Spermatogenesis in *Blasia pusilla*, L. Ann. of Bot., vol. xxvii, p. 93.
- (1915): Spermatogenesis in *Mnium affine*, var. *ciliaris* (Grev.), C. M. Ann. of Bot., vol. xxix, p. 441.
- YAMANOUCHI, S. (1908): Spermatogenesis, Oogenesis, and Fertilization in *Nephrodium*. Bot. Gaz., vol. xlv, p. 145.

EXPLANATION OF PLATES XV AND XVI.

Illustrating Professor Allen's paper on the Spermatogenesis of *Polytrichum juniperinum*.

The figures were drawn with the aid of a camera lucida, the drawing being at the level of the base of the microscope; some of them with a Zeiss 2 mm. apochromatic objective, 1.40 N.A., and compensation ocular number 18; the others with a Leitz 2 mm. apochromatic objective, 1.32 N.A., and compensation ocular number 18. In each case the magnification is about 3800 ×.

PLATE XV.

Figs. 1, 2. Pairs of young sister androcytes, each showing a blepharoplast in the region occupied by a pole of the spindle in the division recently completed.

Figs. 3, 4. Single androcytes; the blepharoplast in each has grown somewhat.

Fig. 5. An androcyte whose blepharoplast has become a short rod; the limosphere also visible.

Figs. 6–10. Stages in the elongation of the blepharoplast; the limosphere visible in Figs. 9 and 10.

Figs. 11–13. Androcytes with still longer blepharoplasts and distinct limospheres. At the upper end of Fig. 13 two unusually large androcytes.

Figs. 14–19. Different views of androcytes all at about the same stage of development; the blepharoplast has become a curved rod, lying in a peripheral position with one end close to the limosphere.

Fig. 20. An androcyte containing a double (or dividing) limosphere; the blepharoplast is not, at least for a considerable part of its length, peripheral.

Figs. 21–4. Stages in the division of the limosphere. The lowermost androcyte represented in Fig. 24 shows an unusual (perhaps abnormal) condition of the limosphere.

Fig. 25. The apical body is now separated from the limosphere.

Fig. 26. The limosphere is moving away from the region in which the apical body was cut off from it.

PLATE XVI.

Fig. 27. The limosphere is in contact with the nucleus; the dark object lying over the apical body is probably the percnosome.

Fig. 28. Beginning of the elongation of the nucleus; the apical body and limosphere are connected by a cytoplasmic strand.

Fig. 29. Different view of an androcyte at about the same stage as that shown in Fig. 28; part of one cilium is visible.

Figs. 30-6. Stages in the elongation of the nucleus; the limosphere is in contact with the posterior part of the nucleus. Except in Figs. 34 and 36, the percnosome appears as a comparatively large body.

Figs. 37-41. Further stages in the elongation of the nucleus; the conspicuous cytoplasmic granule appearing in Figs. 37-9 is probably the percnosome.

Fig. 42. The nucleus has taken on an unusual form, having pushed out two long beaks, one anteriorly and one posteriorly. A part of one cilium appears.

Figs. 43-7. Still further stages in the extension of the nucleus. A portion of the nucleus, near its posterior end, is still comparatively thick. The androcyte shown in Fig. 45, like that in Fig. 41, lies in a vesicle which is bounded by what looks like a definite membrane.

Figs. 48, 49. The nucleus has reached substantially its final length and, by a diminution in its volume, is becoming slender throughout. A vacuole is present in the cytoplasm, as also in Figs. 46 and 47; in this vacuole lies a granule, perhaps the percnosome.

Fig. 50. Two nearly mature androcytes seen in section; the cilia are conspicuous, and some of the cytoplasm remains, though its volume is much reduced, the cell as a whole being lenticular. Each androcyte lies in a vesicle bounded by a membrane.

Figs. 51, 52. Still older androcytes; the cytoplasm is now in two masses, one attached to the anterior, the other to the posterior, portion of the body of the antherozoid.

Fig. 53. The posterior end of the body of an antherozoid at the stage of Figs. 51 and 52, showing the posterior cytoplasmic mass, including the limosphere.

Figs. 54, 55. Antherozoids from sections of mature or nearly mature antheridia. In the antheridium from which Fig. 55 was drawn, the intervesicular substance is stained, but no definite vesicular membrane is visible. In the antheridium from which Fig. 54 was made, the inter-vesicular substance was so faintly stained as to be practically invisible.

Figs. 56, 57. Antherozoids still within their enclosing vesicles, killed after leaving the antheridia. The body shown in Fig. 57, with deeply stained margin and somewhat irregular outline, is probably the limosphere.

Fig. 58. An antherozoid beginning to escape from its vesicle.

Fig. 59. An antherozoid which has become free. Cytoplasmic remnants are still attached to the posterior portion of its body.

Allen, Charles E. 1917. "The spermatogenesis of *Polytrichum juniperinum*." *Annals of botany* 31, 269–291.

<https://doi.org/10.1093/oxfordjournals.aob.a089646>.

View This Item Online: <https://www.biodiversitylibrary.org/item/232813>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a089646>

Permalink: <https://www.biodiversitylibrary.org/partpdf/320189>

Holding Institution

Smithsonian Libraries

Sponsored by

Biodiversity Heritage Library

Copyright & Reuse

Copyright Status: Not in copyright. The BHL knows of no copyright restrictions on this item.

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.