Spermatogenesis in *Blasia pusilla*, L.

BY

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With Plate XI.

IN a recent paper I discussed spermatogenesis in *Porella*, *Marchantia*, and *Fegatella*. The essential features in the development of the sperm were found to be quite similar in these three Liverworts. I came to the conclusion that 'we may consider the mature sperm to represent the two constant cell elements, nucleus and cytoplasm; the main body or nuclear portion representing the nucleus, the blepharoplast and cilia representing specialized parts of the cytoplasm, and the remainder of the latter being found in the cytoplasmic vesicle'. In *Marchantia* and *Fegatella* the last division of the spermogenous tissue results in a pair of triangular-shaped spermatids in each cubical cell. No cellulose wall separates the members of the pair, but each is surrounded by a plasma membrane, and becomes directly transformed into the mature sperm. In *Porella* the last division is not constantly in a diagonal plane, but the fundamental features seem to be the same. The phenomena which have caused the greatest amount of discussion are the origin and nature of the blepharoplast, and the origin and nature of the polar body occurring in certain mitotic stages of *Marchantia*, *Fegatella*, *Riccia*, and certain of the Musci. The various views of the more recent writers were discussed in my former paper. Evidence obtained in my previous studies indicated the origin of the polar or centrosome-like body as a differentiation of cytoplasmic or kinoplasmic materials. It apparently does not possess morphological rank, as its genetic continuity could not be established from an examination of closely consecutive mitotic stages. If such a body does exist from one cell generation to the next its chemical nature evidently changes greatly, so as to cause it, at times, to lose entirely its staining capacity. During metakinesis in spermogenous tissue of *Marchantia*, *Fegatella*, and *Porella*, no centrosome-like bodies were found. During the diagonal division those which occur do not show evidence of a centrosome nature, and do not persist, at least with staining capacity, during telophase. The blepharoplast begins its development in the spermatid as a dark, deeply stained granule in a dense area of cytoplasm. It would seem that

this granule is the result of a concentration or differentiation of cytoplasmic material. No evidence is at hand to show its identity with a previously formed body, or that it is of nuclear origin.

Quite recently Wilson ('11) has published the results of a study of *Mnium* and *Atrichum*, two members of the Musci, and *Pellia*, one of the Hepaticae. The latter is of special interest in this connexion, because of its close relation systematically to *Blasia*. Both belong to the anacrogenous group of the Jungermanniales, thus occupying an intermediate position between *Porella* of the acrogenous division and *Marchantia* and *Fegatella* of the Marchantiales. In *Mnium* and *Atrichum* he describes the nuclear origin of the blepharoplast in the spermatid, the nuclear origin of an accessory body, and the same origin for a very unique structure which he terms a 'limosphere'. The latter is a mass of chromatic material built up from rod-shaped bodies, which have passed out into the cytoplasm from the nucleolus. The 'limosphere' is also present in the spermatid of *Pellia*. No such structure has been discovered in the closely related *Blasia* and *Porella*, or in *Marchantia* and *Fegatella* of the Marchantiales. In other respects a number of Wilson's figures show a striking similarity to corresponding ones of the writer's figures of *Blasia*. In the two Mosses investigated, Wilson finds that the blepharoplast may originate from a probable centrosome, a conspicuous centrosphere being present during certain stages of the ultimate division of the spermogenous tissue. He finds also in all three plants a thread distinct in structure from the blepharoplast connecting the latter with the nucleus. The author has observed in *Polytrichum* appearances somewhat similar to stages of the 'limosphere' described by Wilson for *Mnium* and *Atrichum*, but is compelled to interpret them quite differently. Escaped sperms of *Funaria* killed and stained on the slide show practically the same form and structure as do those of the Hepaticae described by the writer. Figures and discussion of *Funaria* and other Musci will be presented in a later paper.

Allen ('12) has made a careful investigation of the spermogenous tissue of *Polytrichum juniperinum*, including the last division and the resulting pairs of spermatids. These he prefers to term 'androcytes', and the earlier cells of the spermogenous tissue 'androgones'; hence the ultimate androgones function as androcyte mother-cells. The androgones are characterized by the presence of polar plates of kinoplasmic material, or in later cell generations by groups of 'kinetosomes' instead of plates. In either case, previous to cell-division, a single plate or group of kinetosomes divides, forming two, each taking up its position respectively at the pole of the succeeding spindle. In the androcyte mother-cell, instead of a plate or a group of kinetosomes, a single 'central body' of quite similar behaviour occurs. Surrounding the central body are radiating fibres, some of which extend away from the nucleus towards the periphery of the cell. Connected

with similar polar bodies in the corresponding cell generation of *Marchantia* the writer found no radiations extending peripherally. Allen had difficulty in tracing this central body through the succeeding phases of karyokinesis, but found evidence which he considered sufficient to establish its persistence beyond a reasonable doubt. After division is complete a body, the blepharoplast, is apparent in the region formerly occupied by the pole of the spindle and the central body. So far as the writer has observed in the Hepaticae, the first appearance of the blepharoplast occurs in a different part of the cell, as will be shown in the following paper.

It may be of interest to compare these results with those obtained from observations on the *Blasia pusilla*. Antheridial plants were fixed in chromic-osmic-acetic acid at intervals during the month of July. The material, after fixing ordinarily for about twenty-four hours, was washed, brought up through the various grades of alcohol, embedded, and sectioned from 2 to 4 microns thick. Anilin safranin and gentian violet were used as stains, and also Heidenhain's iron alum-haematoxylin counterstained with Bismarck brown. The latter combination seemed to give slightly better results.

CONDITIONS OF GROWTH.

In southern Indiana *Blasia* frequents the banks of muddy streams, often growing on quite high elevations, where it is exposed in the summer months to considerable desiccation. Its habitat is one of fairly constant, though frequently, in times of drought, of rather scant moisture. Mitotic figures were not found to be plentiful, but practically all stages in the development of the spermogenous tissue with the exception of the spindle of the last division were observed. There seems to have been acquired here, as also suggested in the case of *Porella*, a correlation between the conditions of growth and cytological phenomena in the plant's ability to dry up to a certain extent for a period of time, and then revive with rapid cell-division. *Blasia*, however, requires a much more constant supply of moisture than do the leafy Liverworts, but not so much as do the Marchantiales. In the latter, cell-divisions follow each other rapidly, and the spermogenous tissues are, as a rule, brought to maturity without long periods of delay. There is a noticeable difference between the cells of actively dividing spermogenous tissue and those which apparently have been some time in a resting condition. Fig. 1, Pl. XI, represents a stage in which many antheridia are found. The nucleus shows a very faint membrane, enclosing the nucleolus surrounded by a substance very fine and evenly granular. The cytoplasm surrounding the nucleus has very much the same appearance, except that it is somewhat more coarsely granular. In some cells the structures appear to be more or less disorganized and stain very poorly, while others show definite organization. It is important to consider this

fact in connexion with the study of such plants as are so noticeably affected by the pressure or lack of moisture. In order to draw conclusions which are not the result of observation of artifacts, the material should show some signs, at least, of decided activity.

CYTOLOGY OF SPERMOGENOUS TISSUE.

A resting condition of the antheridial cells frequently found is represented in Fig. 1. The cytoplasm is finely and evenly granular. The nuclear cavity surrounding the nucleolus is filled with an extremely fine granular substance, almost homogeneous in appearance. A somewhat lighter space immediately surrounds the nucleolus. The latter is quite sharply defined, and stains very dark and homogeneously. This condition seems to be typical of the tissues after remaining a considerable length of time in a resting condition. As the active conditions arise, and the nucleus passes through the prophases of division, a fine network appears around the nucleolus, with lumps of chromatin of very irregular size, which form at the intersections of the threads. A definite distinction could not always be made between chromatin and linin, the former being drawn out gradually into finely granular threads, forming a network in which the chromatin seems embedded (Figs. 2 and 3). The nucleolus stains more lightly as the chromatin lumps increase in size and finally disappears, as is the general rule during metakinesis (Figs. 2, 3, and 5). The nucleolus is normally present, except immediately preceding metakinesis and during early telophases. It may reappear in the spermatid soon after the last division (Fig. 10), where it remains differentiated only for a short time if the spermatids are in active development. The finely granular substance of the nucleus passes first into a delicate network, and then the threads of the network seem to be absorbed by the chromatin lumps as the latter increase in size and irregularity (Figs. 2, 3, and 5). A definite spireme has not been observed, but very clearly defined chromosomes result, either from such a formation or from the coalescence of the chromatin lumps. Because of the small number of dividing nuclei observed, an exact chromosome count was not obtained, but Fig. 4 suggests five or six as the probable number. In this and similar mitotic figures no evidence of centrosome-like bodies could be found.

FORMATION OF SPERMS.

The last division, like the previous ones, evidently occurs very quickly, judging from the number of stages found immediately preceding and following metakinesis and anaphase. Although not yet observed, the position and form of the resulting spermatids show that the spindle of the last division frequently has an oblique position. Fig. 7 follows very closely the last division. The cytoplasm has not yet divided, as is the case in

Figs. 6 and 9. No wall has been observed between the spermatids as figured by Wilson for *Pellia* and Allen for *Polytrichum*, but the cytoplasm separates into equal masses, each of which with its respective nucleus becomes surrounded by a plasma membrane. No definite centrosome-like body or blepharoplast could be found in those stages represented by Figs. 6, 7, and 8. About this time, however, a small area of cytoplasm denser than the rest appears in one end of each spermatid. The nuclei have in the meantime moved somewhat in opposite directions, leaving the cytoplasm massed on one side of each nucleus, or in opposite ends respectively of each of the pairs of spermatids (cf. Fig. 6, 7, and 8). Fig. 8 represents one of a pair of spermatids drawn from the same section as Fig. 6, but viewed from the back or flattened side, if we speak of the view in Fig. 6 as from the edge. The evidence obtained from a careful examination of the stages similar to Figs. 6, 7, and 9, leaves little doubt that the blepharoplast arises as a differentiation of cytoplasmic material, and agrees with facts observed and described for *Porella* and *Fegatella*. A little later, in each spermatid in the region where the dense area of cytoplasm appeared (Fig. 6) there is found a definite granule or body (Fig. 9). The different writers are quite agreed upon the appearance of this body in the early stages of the spermatid, but not upon its origin and nature. While Wilson does not trace it through the various stages of the last division, yet he thinks it probably arises from a centrosome-like body.

Allen concludes that this is the same morphological entity as the 'central body' which often appears so prominent during the earlier phases of karyokinesis in the 'androcyte' mother-cell.

The results of the writer's observations in regard to the persistence of this body throughout the various phases of the last division of the spermatogenous tissue in the Hepaticae have so far been only negative.

The granule, or blepharoplast, as we may call it, since the latter term indicates its function, grows gradually into a cord along or near the periphery of the cell (Figs. 9, 10, and 11). Fig. 11 shows with remarkable clearness the course and appearance of the blepharoplasts in each pair of spermatids. The one on the right shows the blepharoplast as a cord passing from end to end of the spermatid over the nucleus next to the observer, while the one to the left shows merely the two ends of the cord as the middle portion is hid behind the nucleus and cytoplasm. There is good evidence here that the blepharoplast is formed within the plasma membrane embedded in cytoplasm, and is not a differentiation of the former. The blepharoplast, moreover, appears as a homogeneous cord throughout. The author has taken especial pains to observe its development from its beginning as a small round body until a considerable length has been reached (as in Fig. 11), and no secondary cord has been observed joining the blepharoplast to the nucleus, as one or two writers have recently

described in the Bryophytes, Wilson in *Mnium*, *Atrichum*, and *Pellia*, and Ikeno in *Marchantia*.

In order to understand fully the development of the sperm it is well at this point to compare the views we have in the various figures. Figs. 6, 7, 9, and 11 are corresponding views, all being drawn in a plane at right angles to the plane of the last division, while Figs. 8, 10, 12, and 13 each represent a plane parallel to that of the last division.

The blepharoplast following near the plasma membrane develops into the shape of a comma and approaches a circle. The nucleus in the meantime grows more homogeneous in appearance (Figs. 10, 11, and 12), and becomes crescent-shaped and more closely applied to the blepharoplast. After the stage represented by Fig. 12, it becomes quite difficult to distinguish between blepharoplast and nucleus. The latter seems to increase slightly in size as it becomes more drawn out, and extends from a crescent to a circular form. In Fig. 13 the sperm has lengthened out until it makes somewhat more than one complete turn within the mother membrane. The cytoplasm, which was at first collected within the concavity of the nucleus, eventually disappears, much of it evidently going towards the increase in size of the body of the sperm.

In the stage represented by Fig. 13, the ends of the sperm overlap so closely that it is often difficult to distinguish the two, so that from an observance of this stage only, one might be led into the error of thinking the sperm a complete and closed ring. It is only by following it through its developmental stages that its true nature can be ascertained. M. Leclerc du Sablon ('88) describes a similar picture for *Metzeria furcata*. In speaking of the developing sperm, he says: 'Around the periphery of the cell a thin thread of cytoplasm differentiates, forming a complete circle. The nucleus comes into contact with this thread, . . . becomes smaller and the cytoplasm less dense. The thread or filament seems to be made up from nucleus and cytoplasm. Finally, the nucleus seems to disappear entirely, its substance having been used up to form the sperm. One can see only a colourless vacuole in the centre of the ring formed by the sperm. . . . Soon the ring breaks, the filament thins and lengthens, and gradually acquires the form of the mature sperm.' No figures accompanied M. Leclerc du Sablon's paper, but his descriptions lead one to believe that the figures, if drawn, might have compared quite closely with those of *Blasia pusilla*, as shown by Figs. 12 and 13.

No mature sperms from the antheridium were obtained, but Fig. 14 shows the form frequently found in sections. It is very difficult to obtain them in this way with the cilia intact. These are so long (Fig. 14 shows one approximately 58 microns in length, although they vary much in size), and coiled together in such a manner that the knife usually cuts them at some point or other. In antheridia, however, which are full of mature

sperms, sections or fragments of the cilia are found in abundance, indicating form, position, and number similar to those for other described Hepaticae.

SUMMARY.

The mitotic stages in the spermatogenous tissue show no indications of centrosomes, the divisions occurring in the usual manner.

The spindle of the last division may be placed obliquely, but this does not seem to be always the case. A pair of spermatids, however, results from each cubical cell. An individual membrane surrounds each spermatid, but no wall separates the two, as is the case in *Polytrichum* (Allen, '12).

The blepharoplast makes its appearance first as a dense area of cytoplasm in opposite ends, respectively, of each of the pair of spermatids. Gradually a definite granule or body is differentiated, which develops as a thread or cord around the cell near to the plasma membrane. This cord, the blepharoplast, stains homogeneously throughout. Following its course the nucleus lengthens in close contact with the blepharoplast, the two become indistinguishable by the time one complete turn is made, and the body of the sperm which stains like chromatin continues to increase in length until the mature form is reached (Fig. 14). Two cilia are developed, probably, from the forward end of the blepharoplast.

No accessory bodies of any sort corresponding either to the 'Nebenkörper' of Ikeno or the 'limosphere' of Wilson are differentiated.

DISCUSSION.

It is of some interest to note how similar the phenomena are which occur in the spermatogenesis of various Liverworts. However, we would expect to find this similarity in the reproductive cells of plants so closely related. Possibly the main point of difference between certain ones—*Blasia* and *Porella*, for instance—is found in the larger size of the sperm body of the former. A larger amount of chromatin, however, seems to exist in the spermatid, and in those stages which lead up to the mature form. The mere matter of size does not seem to be a fundamental difference. A phenomenon of more importance is the appearance of a polar body during the diagonal division in *Marchantia*. But if this body does not have morphological continuity, as my observations led me to believe, there is some question as to how much importance should be attached to it, unless an interpretation be placed upon it different from that given by former writers. It is of interest to recall the fact that of the thallose Hepaticae, *Marchantia* possesses the most highly differentiated gametophyte as regards its general morphology, histology, and the occurrence of centrosomes in the vegetative tissue (Van Hook). The last-named cytological phenomenon, together with the appearance of polar bodies in the metakinesis of the last division of the spermatogenous tissue, may be a concomitant of the morphological and histological differentiation.

Certain developments in *Pellia*, *Mnium*, and *Atrichum*, described by Wilson, form a very striking contrast to those which take place in forms very closely related, at least to *Pellia*, such as *Blasia* and *Porella*. One might expect to find somewhat varying developments on passing over into the Musci. Wilson did not figure the mature sperm in any of the three plants discussed, but the writer finds little difference between the mature sperms of *Funaria* and those of the Liverworts previously described and figured (Fig. 15).

The sperm of the Bryophyta is a model of simplicity and adaptation, and the mature form, which is so admirably suited to the conditions to be met and functions to be exercised, is reached by a very direct and yet gradual transformation. The blepharoplast apparently arises *de novo* as the result of the differentiation of active cytoplasm in the cell in which it is to function. It seems quite probable that the blepharoplast represents not only a cilia bearer, but also a formative portion or region of the protoplasm, which in some manner directs the growth of the spermatid as regards form. It seems quite probable also that, in some cases at least, the actual bulk of the chromatin may be increased at the expense of the cytoplasm. The evident results, of vital relation to the plant's economy, reached by the transformation of the spermatid into the mature form of the sperm, seem to be the preparation of as large an amount of chromatin as possible to be passed into the egg-cell, in a form best adapted to the movements necessarily executed in reaching the latter as quickly as possible. The contour of this form is mapped out by the developing blepharoplast; the form of the nucleus becomes moulded accordingly, and the larger part, if not all, of the cytoplasm is used up in the production of energy for the processes going on, or else become differentiated, adding to the nuclear material of the blepharoplast. There is good evidence that quite a large portion goes to the development of the latter. There seems to be no evidence, in the material I have examined, of chromatin passing out into the cytoplasm. We would hardly expect such to be the case as the nucleus seems to increase rather than decrease in size and staining capacity.

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EXPLANATION OF FIGURES IN PLATE XI.

Illustrating Mr. Woodburn's paper on Spermatogenesis in *Blasia*.

Figs. 3, 4, 7, 8, 9, 11, 12, 13, and 14 were drawn with the aid of the camera lucida and with a Leitz apochromatic 2 mm. objective, 1.30 apert. with compensating ocular 18. $\times 3,000$.

Figs. 1, 2, 5, and 10 were drawn with the aid of the camera lucida and a Spencer achromatic 1.5 mm. objective and Bausch and Lomb $\frac{1}{2}$ " ocular. $\times 2,000$ at table level.

Blasia pusilla.

Fig. 1. A cell of spermatogenous tissue in a resting condition.

Fig. 2. Nucleus of a spermatogenous cell in early prophase of division.

Fig. 3. A slightly more advanced stage than Fig. 2, showing the condition of the cytoplasm.

Fig. 4. Anaphase.

Fig. 5. Spermatogenous cell just previous to last division. The membrane has drawn away from the cell-wall.

Figs. 6 and 7. Pairs of spermatids immediately following the last division, showing different conditions of the chromatin, and the dense areas of cytoplasm where the blepharoplast will appear.

Fig. 8. Spermatid in the same stage of development as in Figs. 6 and 7, but viewed from a different angle. The section lies in the same plane as that of the last division.

Fig. 9. Pair of spermatids, showing the blepharoplast organized as a definite granule. The cytoplasm represented in Fig. 5 has divided.

Fig. 10. Single spermatid. Blepharoplast beginning to lengthen; viewed from the same angle as Fig. 8.

Fig. 11. Pair of spermatids, showing the course of the blepharoplast.

Fig. 12. Sickel-shaped spermatid in about the same stage of development as in Fig. 11, viewed from the same angle as in Fig. 10, showing nucleus and blepharoplast in close contact.

Fig. 13. Sperm becoming coiled within the spermatid membrane.

Fig. 14. Mature sperm as found in antheridium. The cilia have become detached.

Fig. 15. Mature escaped sperm of *Funaria*, showing blepharoplast and cilia.

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WOODBURN—SPERMATOGENESIS IN BLASIA.

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