Spore Formation in *Derbesia*.

BY

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With Plates I and II.

THE genus *Derbesia* described by Solier ('47) is chiefly remarkable for its zoospores. These are very large, and each is provided with a circle of numerous long cilia at its forward end. The general morphology of *Derbesia* is that of the Siphonales, with many points of resemblance to *Bryopsis*, except that the filaments are sparingly and irregularly branched. The zoospores of the Siphonales, however, are biciliate, *Vaucheria* excepted, and small. The peculiarities of the zoospores of *Derbesia* have led Blackman and Tansley ('02) to suggest that its affinities are widely different from *Bryopsis*, a point which will be considered later in the paper.

The zoospores of *Derbesia* offer, then, a very interesting subject for cytological study, since their large size and the peculiar arrangement of the cilia give promise of interesting details in the form and development of the blepharoplast. This interest is further enhanced by Berthold's account of their development. The zoospores are almost invariably uninucleate at maturity, but they are developed in relatively small numbers in sporangia which contain many thousands of nuclei when they are first formed from the parent filaments. Berthold ('81) reported that the larger nuclei, finally present one in each zoospore, are formed by the successive fusions of the very numerous nuclei within the sporangium. This is, I think, the last account of nuclear fusions of this character which has not been disproved by detailed cytological investigation, for similar accounts of nuclear fusions during oogenesis in the Saprolegniales, Peronosporales, and *Vaucheria* have been shown by later research to be incorrect, and such reductions in the number of nuclei within multinucleate reproductive cells have proved to be due in every case to nuclear degeneration rather than to nuclear fusions.

This paper will describe the development and germination of the zoospores of *Derbesia Lamourouxii* (Agardh), Solier, with special reference to the structure and behaviour of the nuclei, and the development and fate of the blepharoplast. The material was gathered in the spring of 1904 at Naples, where, at the Zoological Station, I occupied a table of the Carnegie Institution.

The most satisfactory fixation was obtained with a weak chromo-acetic formula as follows:—1 per cent. chromic acid 25 c.c., 1 per cent. acetic acid 10 c.c., 1 per cent. osmic acid 5 c.c., sea water 60 c.c. A similar formula, with the omission of osmic acid, was also satisfactory. Sections were generally cut 3μ thick. The best stain proved to be iron-alum haematoxylin with congo red, and haematoxylin alone for the details of nuclear structure. It proved somewhat difficult to use safranin and gentian violet because of the numerous plastids which take these stains with avidity.

THE HABITS OF THE ZOOSPORES.

The zoospores are formed slowly, and several days may elapse after the development of the sporangium before the segmentation of the protoplasm begins. The first large crop generally appeared two or three days after material of *Derbesia* was brought into the laboratory and left in sea water, after which zoospores continued to be formed for several days. Attempts to hasten their development by raising the temperature of the water or by diluting it resulted in the death of the plants.

The zoospores in the Naples material were remarkably variable in size and in number, ranging from 30–50 in the smaller sporangia to perhaps 200–300 in the larger. They are spherical or oval, and swim slowly through the water with the circle of cilia forward (Pl. I, Fig. 1). They contain very large numbers of small disk-shaped chloroplasts distributed throughout the protoplast, so that the zoospores are uniformly green and the ciliated region is not conspicuously lighter in colour; there are no pigment spots.

The zoospores come to rest on the region of the protoplast within the circle of cilia, and the latter become distributed in a radiating arrangement (Fig. 2) over the surface to which the zoospores become attached.

THE DEVELOPMENT OF THE SPORANGIUM.

The sporangia are globular structures arising from the sides of the filaments. It must require a number of days for them to reach maturity. The contents of the developing sporangium remain connected with the parent filament by a broad strand of protoplasm until the structure has attained full size (Fig. 3). The strand then becomes narrower by the

gradual formation of a thick ring at the base of the sporangium. Sections of this ring (Fig. 4) show that its substance (evidently cellulose in character) is laid down in concentric layers on the inside of the wall of the filament at the base of the sporangium. The protoplasm of the sporangium is thus gradually pinched off from that of the filament and is not separated by thin cleavage furrows as in the sporangia of the moulds.

The ring-like deposit thickens and finally becomes a heavy plate of cellulose (Fig. 5), which presents a laminate structure, showing that its substance is deposited in successive layers as the protoplasm in the sporangium and in the filament withdraw from one another. The wall shown in Fig. 5 is from a sporangium in which the protoplasmic cleavage to form the spores had already begun.

THE DIFFERENTIATION OF THE NUCLEI IN THE SPORANGIUM AND THE DEGENERATION OF THE SMALLER NUCLEI.

The nuclei which enter the developing sporangium are similar and all of about the same size and a little larger than the plastids (Fig. 6). Each contains a small deeply-staining nucleolus which frequently lies within a clearer circle. The remainder of the nuclear cavity is almost wholly occupied by a large body which stains lightly but is readily distinguishable. This body is probably chromatic in character as shown by its later history, but at this stage it is homogeneous and gives no indication of granular or fibrillar structure. There must be several thousand of these nuclei which enter the developing sporangium.

The nuclei begin to show a differentiation in size very shortly after the protoplasm of the sporangium becomes separated from that of its parent filament or even before this time. Certain of them increase in size and become conspicuous in the sporangium, finally reaching a diameter 4-6 times the size of the plastids (Fig. 8). The large nuclei may have smaller nuclei very near them, singly or in groups, to which they appear in sharp contrast (Fig. 7). The internal structure of the nucleus does not apparently change during its growth. The large homogeneous chromatin body, however, increases in size.

There are, however, marked changes in the appearance of the protoplasm around the enlarging nuclei. A granular cytoplasm forms an envelope so that the plastids come to lie at some distance from the nuclear membrane (Fig. 9), and delicate protoplasmic strands radiate out from the membrane between the plastids into the surrounding protoplasm (Fig. 9). These strands end in deeply-staining granules just outside of the nuclear membrane. The large nuclei are thus very prominent in the sporangia because of their size and the radiating strands from the enveloping zone of granular protoplasm bordered by the plastids.

The larger nuclei are distributed rather uniformly throughout the protoplasm within the sporangium, and they lie generally at some distance from one another. The protoplasm between is vacuolate and filled with plastids among which the smaller nuclei lie singly or in groups (Fig. 7). These nuclei at this time are of about the same size as when the protoplasm entered the developing sporangium, that is, about the size of the plastids, but some of them are a little larger and some smaller. They show a marked tendency to become massed in groups (Fig. 10) in regions of granular cytoplasm free from plastids.

There is no evidence that the smaller nuclei fuse with one another to form the larger. This point was studied with great care. These nuclei decrease in size, and shortly become much smaller than the plastids (Fig. 10). Although they may lie so close together as to be in actual contact, they never unite. The small nuclei are not easily found when they have become half the size of the plastids, but careful search will reveal them in larger and smaller groups and also singly, and the fully mature sporangium, shortly before spore formation, has probably just as many nuclei as when first formed. The small nuclei finally break down and are generally completely lost at the time when the spores are formed, except for deeply-staining globules, which are probably the remains of the nucleoli.

It is difficult to understand the reasons for this rapid differentiation of the nuclei in size. I was not able to discover any cytoplasmic structures, such as coenocentra, which could be interpreted as centres of dynamic or metabolic activity affecting the nourishment of nuclei in their vicinity, as appears to be the case during oogenesis in the Perisporiales and Saprolegniales. However, the arrangement of the protoplasm around the larger nuclei is very suggestive of important dynamic or metabolic relations between them and the cytoplasm in the immediate vicinity. The envelope of granular cytoplasm, which has the appearance of kinoplasm, and more especially the radiating protoplasmic strands, give to the nuclei the appearance of being themselves important centres of dynamic and metabolic activity. Their distribution also at relatively wide intervals throughout the protoplasm indicates that each is really the centre of a definite region of protoplasm in the sporangium. It is probable that the radiating strands are the paths of protoplasmic streams which pass to and fro between the large nuclei and the surrounding protoplasm. They are, therefore, probably the paths of dynamic and metabolic activities between the large nuclei and the regions of the protoplasm which they dominate.

It would seem as though there were an actual struggle for existence among the nuclei in the sporangium to control a limited amount of protoplasm with limited metabolic and dynamic possibilities, and that relatively few of the nuclei were successful while the majority perished. I have already suggested this explanation to account for the degeneration of the

nuclei in the oogonia of the Saprolegniales, Peronosporales, and *Vaucheria* (Davis, '03, p. 341, and '04). The physiological conditions within the oogonia of these groups and the sporangium of *Derbesia* are very similar. These reproductive organs are overstocked with nuclei in the beginning of their development, for reasons that are probably phylogenetic, and related to times when the organs developed many more reproductive cells than they do at present. After the reproductive organs are separated from the parent filaments the nuclei find themselves under conditions where there is not sufficient nourishment for all, and a struggle for existence develops with the subsequent degeneration of all the nuclei except the favoured few.

In the Saprolegniales and Peronosporales the survival of certain nuclei is determined by their favourable position near those metabolic centres in the cytoplasm, the coenocentra. In *Vaucheria* (Davis, '04) the surviving nucleus lies near the centre of the oogonium in an accumulation of cytoplasm which is probably the region of the cell most favourable for nuclear metabolism. The sporangium of *Derbesia* is so large a coenocyte that its cytoplasm would not be expected to become uninucleate. However, regions become differentiated and dominated by certain nuclei which find there more favourable conditions for growth, and consequently obtain a lead over all others, finally forcing their degeneration. The positions of these favoured centres of metabolic activity are marked by the accumulation of granular cytoplasm around the large nuclei and the protoplasmic strands radiating out among the plastids.

The last stages in the degeneration of the small nuclei are more easily followed in *Derbesia* than in *Saprolegnia*, *Albugo*, or *Vaucheria*, because the nuclei are not so minute as in the latter forms. The nuclei decrease in size as shown in Fig. 10 until they are less than half the diameter of the plastids. The nucleolus still stains distinctly, but the chromatin body becomes faint and is finally quite lost, so that the nucleolus alone comes to lie in a vacuole which is the remains of the nuclear cavity. The boundary of the vacuole finally breaks down, and the nucleolus then passes into the cytoplasm as a deeply-staining globule. Fig. 11 shows a group of degenerating nuclei in a region of granular cytoplasm surrounded by plastids. This group is in an advanced state of degeneration. Some of the nuclei still have the nuclear membrane, but the globules lying freely in the cytoplasm are from those that have become disorganized. These nucleoli fragment into smaller globules, which finally disappear.

THE SEGMENTATION OF THE PROTOPLASM.

The segmentation of the protoplasm does not begin until the process of nuclear degeneration is practically ended. Traces of the smaller nuclei may still be found, but they are very difficult to recognize. The sporangium then contains only the larger nuclei quite uniformly distributed through the

protoplasm. Segmentation takes place by cleavage furrows which start at the periphery of the protoplast within the sporangium and cut into the protoplasm in the form of curved and branching furrows (Fig. 12).

The cleavage furrows first mark out fairly large areas which may contain a number of nuclei, but the areas become successively smaller as new furrows are formed at the periphery or strike off from the sides of the older into the protoplasm. Finally, the furrows so divide the protoplasm that it becomes blocked out into approximately equal, uninucleate masses (Fig. 13), and these gradually round up as the zoospore originates. The zoospores are almost invariably uninucleate, but I have found binucleate examples (Pl. II, Fig. 27) with blepharoplasts fully developed. These undoubtedly result from conditions similar to the formation of bi- and tri-nucleate eggs of *Saprolegnia* and some species of *Albugo* (Davis, '03, pp. 243 and 324), i. e. two or more nuclei have been able to exist sufficiently near one another to be included in the same area of protoplasm when the reproductive cells were formed.

The plastids in the zoospore-origins and young zoospores (Fig. 14) are arranged about the nuclei with their longer axis radiating outward, so that the nucleus has very conspicuously the appearance of being the centre of the cell not only geometrically but also dynamically, as shown by the finer structure of the protoplasm. The radiating strands from the cytoplasm investing the nucleus are prominent, each with a granule as its base. The protoplasmic strands run out into the cytoplasm between the plastids, but the latter are so densely crowded that it is impossible to trace the strands far, and I was not able to determine whether they reach the periphery of the zoospore and thus form a radiating mesh between the nucleus and the outer plasma membrane. It seems probable, however, that they do so.

THE DEVELOPMENT OF THE BLEPHAROPLAST AND ITS CILIA.

The blepharoplast of the zoospore of *Derbesia* is at maturity a remarkable structure. It consists of a double ring (Fig. 22) situated just underneath the plasma membrane. The cilia are developed from the lowermost of the two rings and radiate out so as to form a circle or crown at one end of the zoospore (Fig. 1). The blepharoplast is generally about one and a half times or twice the diameter of the nucleus. The study of such a large and remarkably shaped structure presented exceptional opportunities for the investigation of some disputed points concerning the origin and relation of this cilia-forming organ to the nucleus and the outer plasma membrane of the cell.

Previous to the formation of the blepharoplast the nucleus moves from near the centre of the zoospore-origin towards the periphery. It is then clear that the radiating strands on the side of the nucleus nearest the

periphery are actually connected with the outer plasma membrane (Fig. 15). About one-third or one-fourth of these strands may be so connected, while the remainder, as before, radiate out into the cytoplasm between the plastids.

At this time granules may be found on the strands at various distances between the nucleus and the plasma membrane (Fig. 15). The sharpness of the granules indicates that they are organized bodies travelling along the strands, which become arranged in a funnel-shaped form with the broadest region of the funnel at the periphery of the cell. It is, however, possible that what appear to be granules are in the living cells merely thickenings of the strands, and that material moves outwards from the periphery of the nucleus through the strands in a semifluid condition and not as granules; but the sharpness of form and depth of staining of these structures indicate that definite granules are actually present. The appearance of the funnel-shaped arrangement of the strands in relation to the nucleus when viewed from the interior of the zoospore is shown in Fig. 16. The granules accumulate in a circle just underneath the plasma membrane where they later form the blepharoplast. The funnel-shaped group of strands connecting the nucleus with the periphery seems to exert a pull on the plasma membrane so that it is drawn inward as a slight depression or shallow groove.

The granules gradually appear more numerous in the circle just underneath the plasma membrane and with the nucleus lying below (Fig. 17). A portion of such a circle, viewed from the interior of the zoospore, is shown in Plate II, Fig. 18. The granules then fuse with one another to form a ring, which becomes the blepharoplast, staining very black with haematoxylin, and so firm in texture that it may be sharply cut with the microtome knife. Its appearance when viewed from the surface of the zoospore is shown in Fig. 19. The strands connecting the nucleus with the developing blepharoplast become less conspicuous and finally disappear after the blepharoplast is formed, leaving the boundary of the nucleus nearest to the plasma membrane without conspicuous radiations. The nucleus then passes from the periphery back to the centre of the zoospore, and the plastids become distributed around it as before in a radiating arrangement (Fig. 20). The plastids also encroach into the region of the zoospore just under the blepharoplast from which they had previously been excluded by the funnel-shaped group of protoplasmic strands.

The blepharoplast when first formed is a single ring as shown in cross-section (Fig. 21 *b*). It lies very close to the outer plasma membrane, but is not a part of it. The blepharoplast now splits into two rings, one below the other, and the circle of cilia grows out from the lower (Fig. 22). The splitting of the primary ring is a gradual process. The ring changes its form so that in cross-section it appears somewhat U-shaped with the arms of the U very much thickened (Fig. 23 *b*). The two thickened portions then separate

as two rings, one below the other, the lower slightly larger than the upper, so that it appears somewhat outside when the blepharoplast is viewed from above (Figs. 22, 24).

The cilia, as stated before, arise as outgrowths from the lower ring (Figs. 22, 25, 26). It was not possible to determine whether each cilium was connected with a definite granule such as entered into the composition of the blepharoplast, for the latter has the appearance of being an homogeneous structure. It stains evenly, and the granules that formed it (Fig. 18) fuse together so completely that their individuality becomes lost. The splitting of the primary ring is a very curious process, for which I have no explanation to offer.

Mention has been made of the occasional binucleate spore origins. A section of such a one is shown in Fig. 27, so far advanced in its development that its blepharoplast is formed. There can hardly be doubt but that such an example would give rise to a binucleate zoospore. The development of such a zoospore simply means that the progressive segmentation of the protoplasm by the branching cleavage furrows does not always proceed far enough to separate all of the large nuclei from one another.

During the development of the zoospores some important changes take place in the structure of the nucleus. As shown in Fig. 6, the nuclei which enter the sporangium contain two structures: (1) the deeply-staining relatively small nucleolus, and (2) a large homogeneous body which is chromatic in character, as proved by its later history, and which I have called the chromatin body. This latter structure remains unchanged in appearance during the differentiation of the nuclei in size (Fig. 7), but later, changes become apparent within the large nuclei. Delicate strands appear in the nuclear space, forming a loose network (Figs. 8, 10), and the chromatin body ceases to be homogeneous in structure but becomes instead granular or cloudy. Finally, the nuclei at the time of the segmentation of the protoplasm to form the zoospores (Figs. 12, 13) contain loose masses of cloudy material connected by strands to form an irregular network. While the blepharoplast is being developed the network becomes more and more conspicuous and stains more deeply (Figs. 16, 19). The cloudy masses at last practically disappear, and there are present in the nucleus coiled threads, generally somewhat gathered in the centre near the nucleolus (Figs. 21, 24). These coiled threads are clearly chromatic, and from them is organized the spirem of the first mitosis in the germinating spore.

Perhaps the most important result of the present study of *Derbesia* is the clear evidence that the nucleus is concerned in a most intimate manner with the development of the blepharoplast. The nucleus leaves its position in the centre of the cell, the radiating protoplasmic strands over a certain portion of its surface take a definite position in the form of

a funnel, and granules apparently pass outward to the plasma membrane. After the blepharoplast is formed the nucleus returns to the centre of the cell. This important work being accomplished, it is probable that the nucleus maintains connexions with the blepharoplast by very delicate protoplasmic strands, although such conditions would not be easily observed in *Derbesia* because of the numerous and crowded plastids. The characteristic clear areas at the tips of most zoospores of various algae indicate that these regions are occupied by protoplasm, which is kinoplasmic in character, and consequently likely to hold vital relations to the nucleus. It is clear that the blepharoplast of *Derbesia* is not a development from the plasma membrane, but from granules closely associated with protoplasm investing the nucleus.

THE GERMINATION OF THE ZOOSPORES.

As has been stated, the zoospore comes to rest on the region of the protoplast within the circle of cilia, which spread out in a radiating arrangement over the surface to which the zoospore becomes attached (Fig. 2). The spore generally forms a small papilla at this region, which develops into a simple holdfast. The blepharoplast may be easily found during the earlier stages of germination. Fig. 28 (Plate II) shows the base of a germinating spore, the nucleus of which had not yet divided; the blepharoplast appears in section, and it should be noted that the two rings are much more delicate than in the zoospore, although the magnification is twice as great. The nucleus or nuclei in the germinating spores come to lie near the periphery of the cell because of the development of a central vacuole which forces the protoplasm to take position just under the cell wall. Fig. 29 shows the base of an older sporeling which contained several nuclei; the two rings of the blepharoplast are somewhat separated here. In both figures the blepharoplast is evidently pressed very close against the cell wall, which has been formed, and appears now to lie in the plasma membrane. The blepharoplast becomes fainter in older stages of germination, and can be found only with difficulty. It fades away, and probably at last entirely disappears.

The mitoses in the germinating spore are difficult of study because the nuclei are so small. It is, however, very clear that the resting nucleus contains besides the nucleolus a chromatin network (Fig. 30) in place of the chromatin body, which is so characteristic of the nuclei that enter the sporangia (Figs. 6 and 7). This network, as has been shown, develops gradually in the nuclei accompanying the breaking up of the chromatin body into irregular cloudy masses.

The chromatin network changes before mitosis into a spirem, upon which deeply-staining chromatin granules may be easily recognized. I

have seen some evidence of a centre on the exterior of the nucleus (Fig. 31), to which the spirem seemed to be attached, suggesting nuclear polarity similar to that described by Harper ('05) for *Phyllactinia*. However, the conditions in *Derbesia* are not very favourable for the study of this point, and I am not prepared to discuss the matter further at this time.

The spindle at metaphase of mitosis (Fig. 32) is intranuclear, and there is a minute granule at each pole.

CYTOLOGICAL DISCUSSION.

This discussion will concern itself chiefly with the morphology and development of the blepharoplast and its relation to the nucleus. There is a wide divergence of opinion respecting these problems. Strasburger ('92, '00), from studies of zoospores, chiefly those of *Oedogonium*, *Cladophora*, and *Vaucheria*, concluded that in these forms the cilia were derived from a body (blepharoplast) which arose in the outer plasma membrane. This conclusion for *Oedogonium* is of especial interest, since the zoospore of this alga is provided with a circle of cilia similar to that of *Derbesia*. Mottier ('04) has described the blepharoplast of *Chara* as arising in the plasma membrane in agreement with Strasburger's views.

Timberlake ('02) noted fundamentally different conditions in his study of zoospore formation in *Hydrodictyon*. He described the blepharoplast as a body distinct from the plasma membrane and connected with the nucleus by delicate fibres. There is also a period during zoospore formation when the nucleus lies very close to the cleavage furrow, and at this time a granule may sometimes be observed at the side of the nucleus. Timberlake did not trace the origin of the blepharoplast, but it seems very probable that it is derived from this granule close to the nucleus, and remains connected with the latter by delicate fibres, at least during the earlier periods of zoospore formation. There is thus an apparent agreement between *Hydrodictyon* and *Derbesia* in the intimate relation of the blepharoplast to the protoplasm around the nucleus and its independence in origin of the outer plasma membrane.

The other studies on the blepharoplast of plants have been concerned chiefly with its possible relation to a centrosome. Belajeff in a series of papers ('97 *a*, '97 *b*, '97 *c*, '98, '99) described the blepharoplasts of *Gymnogramme*, *Equisetum* and *Marsilia*. He found the blepharoplasts at the poles of the spindles in the mitosis just preceding the formation of the sperms and regarded them as centrosomes. Shaw ('98) held that the blepharoplasts of *Marsilia* did not occupy the poles of the spindle of the final mitosis, but were formed at the poles of the preceding spindle; Belajeff, however, disputes this.

Ikeno ('98) for *Cycas*, Hirase ('98) for *Ginkgo*, and Webber ('01) for *Zamia* agree in describing the blepharoplasts as arising *de novo* on opposite sides of the nucleus and at some distance from it, before the mitosis that precedes the differentiation of the sperm nuclei. The spindle of this mitosis is said to hold no relation to the two blepharoplasts which are far removed from its poles. Ikeno and Hirase have from the first held that the blepharoplast was an attractive sphere or centrosome, and Ikeno ('04) has more recently emphasized his views of the homology of the centrosome and blepharoplast of plants. Webber, however, laid stress on the striking fact that the blepharoplasts are independent of the spindle fibres of the last mitosis of spermatogenesis of *Zamia*, and are formed *de novo* at some distance from this nucleus, while it is in the resting condition, a developmental history which would not be expected of a centrosome-like structure.

Ikeno ('03) has also reported centrosomes during the mitoses of spermatogenesis in *Marchantia*, stating that the centrosomes of the last mitosis become the blepharoplasts of the sperms. Miyake ('05) has not been able to confirm Ikeno's conclusions respecting the presence of centrosomes during the mitoses of spermatogenesis. However, he finds a body at each spindle pole of the last mitosis of *Marchantia* and groups of granules similarly situated in *Makinoa*, and he considers these as likely to function as blepharoplasts. Ikeno ('05) has replied to the criticisms of Miyake, but this discussion is not essential to the problem before us.

The views of botanists respecting the origin and nature of the blepharoplast are then at variance in fundamental points. Strasburger, Shaw, Webber, and Mottier hold that the blepharoplast is not a centrosome, but arises either in the plasma membrane or in the cytoplasm, independently of the nucleus. Belajeff, Ikeno, and Hirase believe it to be related to the centrosome. There are conflicting accounts of its history even in the same forms, and very little information concerning its origin even in the most favourable types, such as the cycads and *Ginkgo*.

Let us now consider the history of the blepharoplast of *Derbesia* in relation to the confusion of views described above. The blepharoplast of *Derbesia* has clearly very important physiological relations to the nucleus. The granules that enter into its composition come from the surface of the nucleus and travel along a system of protoplasmic strands to the plasma membrane *beneath which* the blepharoplast is formed. Timberlake's account of *Hydrodictyon* has many points of agreement with this history and offers substantial support, although the zoospores of that form are too small to trace the development of the blepharoplast as easily as may be done in *Derbesia*. This history for *Derbesia* does not accord at all with Strasburger's view of the origin of the blepharoplasts in *Oedogonium*, *Cladophora*, and *Vaucheria* from the plasma membrane, or the similar account of Mottier for *Chara*. I cannot but believe that a closer study of the development of the

blepharoplast in these types will show physiological connexions between this structure and the nucleus, and that the blepharoplast will be found to arise, not from the plasma membrane, but from material associated with the nucleus. The zoospore of *Oedogonium* will probably prove very favourable for investigation from this point of view.

With respect to the theory of a relationship between the blepharoplast and centrosome, *Derbesia* furnishes clear evidence in the negative. In this type the blepharoplast is formed from a very large number of granules derived from perhaps a third or a fourth of the nuclear surface, all similar to one another and to the other granules at the bases of the numerous radiating protoplasmic strands. These granules cannot be centrosomes although they may be kinoplasmic in character. I think it is possible that the centrosome theory of the blepharoplast, as held by Belajeff, Ikeno, and Hirase, will not receive support when types are studied in which the blepharoplast is not developed in close association with mitosis. The presence of a mitosis immediately preceding the formation of sperms in the liverworts and ferns and the position of the developing blepharoplast at the poles of these spindles naturally lead to its association with a centrosome. There are no mitoses immediately preceding zoospore formation in *Derbesia*, or indeed in the sporangium at all, and similar conditions are also present during zoospore formation in *Oedogonium* and a number of other Algae which should be studied. The investigation of the origin of the blepharoplast in such forms (as also in the Cycads and *Ginkgo*) will probably show that the blepharoplast, in these cases at least, is not the homologue of the centrosome as this structure is generally understood. Nevertheless, it must be borne in mind that the studies on spermatogenesis in animals have shown that the locomotor apparatus of the spermatozoid is formed in large part from the centrosome, and botanists have been fully justified in approaching the investigation of spermatogenesis in plants from that point of view. It is very important that more detailed studies on spermatogenesis in plants be undertaken upon such favourable types as *Marsilia*, certain ferns, the Cycads, and *Ginkgo*.

It is possible that both the blepharoplast and centrosome may prove to have fundamental points of resemblance in their relation to kinoplasm investing the nucleus and to systems of radiating protoplasmic strands running out into the cytoplasm. The substance of the blepharoplast and centrosome may be similar, and their position as dynamic centres may be clearly analogous, but without morphological relationship. There is much evidence that both structures are formed at the point of convergence of radiating protoplasmic strands, which at certain stages of development are likely to prove to be streams of protoplasm bringing material to a dynamic centre. When such streams converge to a well-defined centre the whole system takes the form of an aster. Indeed there are reasons for

believing that the asters associated with mitosis are such dynamic centres, and their radiating fibres either the paths of delicate protoplasmic streams or developments from such streams. According to this view the aster stands as the morphological expression of dynamic activities, and the centrosome and centrosphere are rather the result of these activities, associated with the rays converging to a common point, than the cause of the structure.

The structure of the cilia-forming apparatus in epithelial cells of animals is of interest in relation to this study of *Derbesia*. Earlier views, which held the granules below the cilia to be centrosomal in nature, have been discredited by later work. The subject is discussed in a paper of Wallengren ('05) on *Anodonta*. Wallengren has followed the mitotic division of the nucleus in the epithelial cells from the gills of this mussel. A centrosome (diplosome) is present, lying among the granules at the bases of the cilia, and accompanies the nucleus in its mitosis. During the mitosis the cilia first disappear, followed by the basal granules and the fibrils that extend from them into the cytoplasm. The cell divides by constriction after which a thick protoplasmic layer is formed under the new cuticula. Granules arise in this layer of protoplasm without genetic relation to the mitotic figure. From each of these granules a fibril is first developed extending into the cytoplasm, after which a cilium grows out through the cuticula. The granules, fibrils, and cilia form, all together, the ciliated apparatus corresponding to the blepharoplast of plants with their cilia and internal fibrils, when present.

The significant feature of this history of the development of the cilia-forming apparatus in epithelial cells, in relation to the origin of the blepharoplast in plants, is its independence of centrosomes or other structures of the mitotic figure. The fibrils penetrating the interior of the cell, however, probably hold important physiological relations to the nucleus. Such fibrils are well known in epithelial cells, and have been described and figured as penetrating to the protoplasm immediately investing the nucleus; see discussion of Gurwitsch ('04), p. 67. Their presence indicates that the granules bearing cilia have intimate dynamic connexions with the protoplasm and nucleus of the cell. The blepharoplast of *Derbesia* during its development shows clearly a similar physiological dependence upon the nucleus and cytoplasm, a dependence which probably lasts as long as the cilia are motile, although conspicuous protoplasmic strands connecting the nucleus with the blepharoplast are not evident after the blepharoplast is fully formed.

Studies upon Infusoria have established the presence of granules at the bases of the cilia, which in some forms are, of course, very numerous. Maier ('03) believes that these can hold no possible relation to centrosomes, which are unknown in the Infusoria. These conclusions are thus in accord

with those of Gurwitsch and Wallengren that the granules at the bases of cilia in epithelial cells are not related to centrosomes.

The Flagellata present excellent subjects for the investigation of cilia-forming organs, which in this group have not as yet received the attention that they deserve. Plenge ('99) established the presence of a granule at the base of the cilium in the swarm spore of the myxomycete, *Didymium*, and the connexion of this granule by a delicate fibril with the nucleus of the cell. Similar conditions were also observed by him for several flagellates. Dangeard ('01) has shown in some detail for *Polytoma* that the two cilia arise from a small body connected with the nucleus by a delicate fibril, and Maier ('03), p. 145, confirms his observations, and also reports a similar structure in the cells of *Chlamydomonas*. Prowazek ('03) studied the conditions in a number of flagellates, finding the cilium in some forms in intimate connexion with the nucleus, and in others arising from a granule connected with the nucleus by a fibril, or with its base so connected. His observations thus support those of Plenge, Dangeard, and Maier. All of these authors have discussed the possibility of the basal granule being centrosomal in character when comparing these ciliated cells with the spermatozoan, but definite conclusions on this subject must await detailed investigations on nuclear division and the origin of the cilia in daughter-cells of the flagellates. However, it does not seem probable that the basal granule is a centrosome.

There is another phase of the subject of cilia-bearing organs of animal cells which must be mentioned, since it indicates that such structures may have different origins and developmental histories in different tissues. That centrosomes are intimately concerned with the development of the locomotor apparatus of the spermatozoid seems to be established from the studies on spermatogenesis in animals; see discussions of Meves ('01) and Waldeyer ('06). There is, therefore, a situation with respect to the origin of cilia-bearing organs of animal cells apparently somewhat similar to the divergent accounts of plant cells. That is, these organs have a relation to centrosomes in the spermatozoid but an independence of centrosomes in epithelial cells, in those of Infusoria and probably also the Flagellata. Whether the blepharoplast of the plant sperm is related to the centrosome is, however, a matter demanding further investigation.

THE ZOOSPORE AND MOTILE GAMETE AS TAXONOMIC CHARACTERS IN THE SIPHONALES.

Within recent years emphasis has been laid by a number of writers upon the zoospore and motile gamete as structures of fundamental importance in determining the relationships of great groups among the algae. The motile reproductive cells are held to retain in the various groups the

characters of a primitive ancestry from forms allied to the flagellates. In other words the periods of sporogenesis and gametogenesis are regarded as periods in the life-history when the algae return for a short time to conditions similar to those of their ancestry, and the structure of these motile cells may be expected to give important evidence of the morphology of the flagellate-like types from which various classes and orders of the algae may have been derived.

This view unquestionably has much of value both in practice and theory. However, its application may be carried to extremes, and classifications based chiefly or wholly on the zoospores and motile gametes are likely to prove artificial and open to criticism, after the manner of many systems which have been proposed on single characters. We have in the Siphonales an assemblage of algae which illustrate very clearly the danger of attaching too much importance to these reproductive cells as taxonomic characters, and *Derbesia* is of especial interest as a test case.

Blackman and Tansley ('02) in their revision of the classification of the green algae have removed *Botrydium* and *Vaucheria* from the Siphonales because the motile reproductive cells do not conform in structure to the zoospores and gametes typical of this group. Although they still retain *Derbesia* because of its general resemblance to *Bryopsis* their opinions are strongly expressed in the following remarks (p. 25 of the reprint): 'Though the vegetative characters of this genus suggest a position among the lower Siphoneae in the neighbourhood of *Bryopsis*, yet the peculiar zoospores, recalling those of *Oedogonium*, probably indicate that its affinities are widely different. Our present information is not however sufficient to justify the removal of the genus from its traditional place.'

An analysis of the process of spore formation in *Derbesia* shows at once how unsafe it would be to conclude that the peculiar structure of its zoospores has deep phylogenetic significance. The early stages of spore formation are those common to all of the Siphonales (including *Vaucheria*); the sporangium becomes filled with multinucleate protoplasm. Then in *Derbesia* there begins the peculiar process of nuclear differentiation and the degeneration of the greater number of smaller nuclei. The process of nuclear degeneration is very exceptional, although finding its analogy during oogenesis in *Vaucheria*, *Saprolegnia*, and in the Peronosporales. The result is the development of a set of extraordinarily large nuclei which become the centres of spore formation. The kinoplasmic activities concerned with these large nuclei produce blepharoplasts of remarkable form and size, capable of developing and bearing a great many cilia instead of the typical number two.

There can be little doubt that the peculiarities of the zoospore of *Derbesia* are later developments in phylogeny concerned with the exceptional features of the process of sporogenesis, and that its structure is not

that of ascestral forms and can have no value in phylogenetic considerations. The evidence indicates that the ancestors of *Derbesia* produced immense numbers of small zoospores represented by the very numerous nuclei which enter the sporangium, and it is very probable that these zoospores were similar to the biciliate elements characteristic of the *Siphonales*.

The zoospore of *Oedogonium* has a structure similar to that of *Derbesia* with respect to the arrangement of its cilia, and I venture to predict that the development of its blepharoplast will be found to parallel closely that of *Derbesia*, for the reason that the zoospore is developed from an exceptionally large uninucleate protoplast. However, I hardly think that any one for these reasons will be bold enough to suggest a relationship between *Derbesia* and *Oedogonium* in view of the striking differences at all points in their general morphology.

Derbesia may serve as a warning of the danger of carrying too far the practice of basing classifications of the algae on the structure of the zoospores and motile gametes. The removal by some authors of *Vaucheria* from the *Siphonales* seems to me open to serious criticism, and has not been followed by other authors, as, for example, Oltmanns ('04). *Botrydium* is of course a peculiar type of uncertain position. This study of *Derbesia* shows clearly the necessity of treating exceptional forms of algae separately, deferring judgement on the significance of peculiarities of zoospores and motile gametes until their development and structure is understood in comparison with these elements in groups of supposed relationship.

SUMMARY OF THE INVESTIGATION OF DERBESIA.

The protoplasm in the globular sporangium becomes separated from that of the parent filament by the formation of a heavy cellulose plate, which begins to develop as a ring at the periphery, and consists of layers deposited successively as the protoplasm in the sporangium and filament withdraw from one another.

The protoplasm within the developing sporangium contains several thousand nuclei. These are all similar to one another, and slightly larger than the plastids at the time when the cross-partition is formed at the base of the sporangium. Each nucleus contains a small nucleolus and a large chromatin body.

A process of nuclear differentiation sets in very shortly after the protoplasm of the sporangium becomes separated from that of the parent filament. Certain nuclei increase in size, reaching a diameter 4-6 times that of the plastids. These larger nuclei also become conspicuous because of the development of numerous protoplasmic strands which radiate out from the cytoplasm enveloping the nucleus. The strands have deeply-staining granules where they join the enveloping cytoplasm just outside of the

nuclear membrane. The larger nuclei become distributed rather uniformly throughout the sporangium, lying generally at some distance from one another.

The smaller nuclei are found sometimes singly but more frequently in groups within areas of cytoplasm bordered by plastids. They never fuse with one another. These smaller nuclei gradually decrease in size, becoming much smaller than the plastids, and finally, losing their chromatin content, break down in the cytoplasm. They have generally disappeared completely at the time the spores are formed, except for deeply-staining globules in the cytoplasm which are the remains of the nucleoli.

The large surviving nuclei are not associated with cytoplasmic centres such as coenocentra, but the arrangement of the protoplasmic strands indicates that they are the paths of cytoplasmic streams passing to and fro between the nuclei and the cytoplasm. The large nuclei are, therefore, probably themselves important centres of dynamic and metabolic activity.

The segmentation of the protoplasm does not begin until the process of nuclear degeneration is practically ended. Cleavage begins at the periphery of the protoplast and the protoplasm is quickly cut by curved and branching furrows, that finally divide it into uninucleate masses, which are the zoospore-origins. Occasional binucleate spore-origins have been noted. The zoospore-origins round up and the plastids take a radiate arrangement around the centrally placed nucleus with its investing envelope of cytoplasm and radiating protoplasmic strands.

The nucleus of the spore-origin then moves from the centre of the protoplast towards the periphery. Perhaps a third or a fourth of the protoplasmic strands on the side of the nucleus nearest the periphery become arranged in the form of a funnel. Granules may be found on these strands apparently moving outward towards the plasma membrane.

These numerous granules accumulate in a circle just underneath the plasma membrane and fuse with one another to form a deeply-staining, firm ring, which is the blepharoplast. The strands connecting the nucleus with the developing blepharoplast apparently disappear after its development, and the nucleus passes back to the centre of the zoospore, the plastids becoming distributed about it again in a radiating arrangement.

The blepharoplast then splits to form two rings, one slightly below the other, and the circle of cilia is developed from the lower ring.

During the development of the zoospores the homogeneous chromatin body undergoes changes; its material becomes granular or cloudy and strands appear, forming an irregular network of coiled threads, from which is developed the spirem of the first mitosis in the germinating spore.

The two rings of the blepharoplast remain for some time closely pressed against the cell wall at the base of the germinating spore, but they

gradually grow fainter and probably at last disappear. The nucleus of the germinating spore divides mitotically, the spindle being intranuclear.

It is clear that the nucleus of *Derbesia* has very intimate relations to the development of the blepharoplast, and that the latter does not arise from the plasma membrane, but from granules associated with the protoplasm investing the nucleus. These granules are not, however, centrosomes.

CAMBRIDGE, U.S.A.,

March, 1907.

Note:—Since the above was written there has appeared a paper by Escoyez ('07), 'Blépharoplaste et Centrosome dans le *Marchantia polymorpha*,' La Cellule, xxiv, 247. This author, contrary to Ikeno ('03), finds no centrosomes in the mitoses preceding the differentiation of the sperm-mother-cells. Blepharoplasts, two in number, were first observed in contact with the plasma-membrane in the sperm-mother-cell and they occupy the poles of the spindle of the mitosis within this cell, which precedes the organization of the two sperm-nuclei. Escoyez does not consider the blepharoplast to be a true centrosome. He has not traced its origin, and we do not know whether it comes from within the cytoplasm near the nucleus or is formed in the plasma membrane. The former alternative is to be expected.

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EXPLANATION OF FIGURES IN PLATES I AND II.

Illustrating Dr. Davis's paper on *Derbesia*.

The preparations were studied under the Zeiss apochromatic oil immersion 1.5 mm. with the compensating oculars. The figures were sketched with an Abbé camera from sections generally cut 3μ thick and stained with iron-alum haematoxylin after the method of Heidenhain. The magnification was as follows: Fig. 1, 1000 diameters; Figs. 2, 12, 13, and 27, 667 diameters; Figs. 6-9, 14-26, 28, and 29, 1334 diameters; Figs. 10, 11, and 30-32, 2000 diameters.

PLATE I.

- Fig. 1. Zoospore in side view, stained with iodine to show circle of cilia.
- Fig. 2. Zoospore shortly after coming to rest, viewed from above, stained with iodine.
- Fig. 3. Outline of a sporangium in section, the protoplasm of which is still in communication with that of the parent filament.
- Fig. 4. A stage in the formation of the cross partition at the base of the sporangium; there is present a ring-shaped thickening growing inward from the periphery and showing a laminate structure.
- Fig. 5. The cross partition at the base of a sporangium showing the laminate structure.
- Fig. 6. The nuclei as they enter the sporangium, illustrating their relative size in comparison with the plastids; each nucleus contains a small nucleolus and a large chromatin body.

Fig. 7. Large and small nuclei within a young sporangium before the cleavage of the protoplasm.

Fig. 8. A large nucleus heavily stained, showing the enveloping cytoplasm and radiating protoplasmic strands.

Fig. 9. A large nucleus lightly stained to differentiate the granule at the base of each protoplasmic strand.

Fig. 10. A large nucleus and a neighbouring group of small degenerating nuclei in a region of cytoplasm free from plastids; the latter are now much smaller than the plastids.

Fig. 11. A group of smaller nuclei in an advanced state of degeneration; the nuclear membranes of some have disappeared, and the nucleoli lie in the cytoplasm.

Fig. 12. The beginning of segmentation by branching cleavage furrows cutting into the protoplasm from the periphery.

Fig. 13. Spore-origins rounding up.

Fig. 14. A spore-origin, showing the centrally placed nucleus from which protoplasmic strands radiate among the plastids, whose long diameters generally point towards the nucleus.

Fig. 15. A nucleus lying near the periphery of a spore-origin, the granules apparently passing outward along the protoplasmic strands between the nucleus and the plasma membrane.

Fig. 16. A stage similar to, but slightly older than that shown in Fig. 15. The nucleus and funnel-shaped arrangement of the protoplasmic strands with the granules is viewed from the interior of the spore-origin.

Fig. 17. The granules are arranged in a circle just underneath the plasma membrane.

PLATE II.

Fig. 18. Portion of a circle of granules underneath the plasma membrane, viewed from the exterior of the spore-origin.

Fig. 19. The granules have fused to form a firm ring, the blepharoplast, above the nucleus and just beneath the plasma membrane.

Fig. 20. A spore-origin after the formation and splitting of the blepharoplast ring, showing the nucleus now in the centre of the protoplast and the plastids again arranged in a radiating manner.

Fig. 21. The blepharoplast (*b*) in section before it splits to form two rings. A chromatin network is clearly present in the nucleus.

Fig. 22. The mature blepharoplast, showing cilia developing from the lower of the two rings.

Fig. 23. The splitting of the blepharoplast (*b*), as seen in section, to form two rings.

Fig. 24. A portion of a blepharoplast, showing the two rings.

Fig. 25. The two rings of the blepharoplast, seen in section, with cilia attached to the lower.

Fig. 26. The blepharoplast in section, cilia attached to the lower ring on the right-hand side.

Fig. 27. A spore-origin with two nuclei and a portion of the blepharoplast; this would have developed into a binucleate zoospore.

Fig. 28. Base of uninucleate germinating spore, showing remains of blepharoplast (*b*).

Fig. 29. Base of sporeling containing several nuclei; the rings of the blepharoplast (*b*) still present.

Fig. 30. Nucleus in germinating spore, showing loose linin network with chromatin granules.

Fig. 31. Nucleus in germinating spore, showing a possible much-coiled spirem apparently connected with a centrosome-like body on the exterior of the nucleus.

Fig. 32. Metaphase of mitosis in the sporeling, an intranuclear spindle.

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