Studies in the Dictyotaceae.

I. The Cytology of the Tetrasporangium and the Germinating Tetraspore.

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With Plates IX and X.

IN 1897 I published an account of the discovery of motile antherozoids in *Dictyota*¹. At the Bristol meeting of the British Association (1898) the mode of fertilization was described. The cytology of the antheridia and oogonia and of the apical region of the tetraspore plant was also explained and illustrated by means of figures. It was found that the full number of chromosomes obtained in the apical nuclei of the asexual plants, while in the single division of the oogonium and in the early divisions of the antheridium the reduced number prevailed. This necessitated a more careful search for the actual reduction division. Ultimately it was found to be the first division in the tetraspore mother-cell. Meantime Mottier² in 1898 announced his discovery of centrosomes in *Dictyota*. Subsequently (in 1900³), he published a detailed description of the reduction division, as well as of the second division of the tetraspore mother-cell and of the vegetative mitoses in the thallus-cells. I have worked out the cytology of all the various kinds of cells in the three forms of *Dictyota*, male, female and asexual, and a considerable body of facts has been gathered relating to the natural history of the living plant, both under cultivation and in its natural habitat. As far as cytological evidence is concerned there seems to be no reason to doubt that we have here a clear case of alternation of generations. Publication of the results has hitherto been delayed in the hope of completing the evidence by actual cultivation of the plant from spore to spore. All the numerous attempts made to produce this result have hitherto failed, owing to the difficulty of getting the plant to fruit in cultivation, and it has been thought best not to further delay

¹ Williams, *Annals of Botany*, xi, 1897.

² Mottier, *Ber. d. deutsch. Bot. Gesellsch.*, xvi, 1898.

³ Mottier, *Ann. of Bot.*, xiv, 1900. All subsequent references to Mottier will be to this paper.

publication of the results hitherto obtained. The present paper deals with the sporophyte generation, and the first segmentation of the spore. The cytology of the gametophyte and of fertilization, the very remarkable phenomena associated with parthenogenesis, as well as the natural history of the plant, will be dealt with in subsequent papers.

Mottier has described and figured karyokinesis in the vegetative cells of the thallus. Our results being in general agreement it is not proposed to deal further with them. As, however, he has not described the stalk-cell division or the earlier stages of the reducing division, a detailed account of them will be given here.

THE STALK-CELL DIVISION.

One of the elongated rectangular cells of the thallus begins to swell in all directions, but chiefly outwards, in the direction of least resistance. At the same time the nucleus increases greatly in size. The nucleolus appears granulate, there is a fine reticulum uniformly distributed through the nuclear cavity, and upon it are numerous granules. The axis of the nucleus is as yet parallel to that of the thallus. At each pole there is a curved rod-like centrosome, and radiations extend into, and become merged in, the cytoplasmic reticulum. The chloroplasts stain uniformly; they are much paler than those of the vegetative cells, and at first they are uniformly distributed. Soon, however, the basal portion immersed in the surface layer becomes greatly vacuolated. In the vertical walls separating the cell from the neighbouring ones there are large pits and the vacuoles lie opposite them.

The lateral extension of the cell soon ceases, but it grows rapidly outwards until it is several times the depth of the cortical cell. In cross section the projecting part is nearly spherical, but a longitudinal section shows an elongated, dome-shaped mass. The dense cytoplasm, containing the bulk of the chloroplasts and the nucleus, is aggregated in the swollen, free portion. The axis of the nucleus has now swung round until it is vertical to the thallus, and the distal pole has a very distinct curved centrosome and beautiful radiations; the space occupied by the latter being quite free of chloroplasts (Pl. IX, Fig. 1). A cap of cytoplasm covers the basal pole. From this a cytoplasmic cord extends downwards into the vacuole, where it sometimes branches. The centrosome here is often only distinguished with difficulty and the radiations are completely absent. Stages where the axis of the nucleus is oblique to the thallus are very difficult to find, the change of position probably taking place very quickly. The nucleolus is now large and stains deeply. Sections through it show that it is uniformly fibrillate, or minutely vesicular in texture.

Before the spirem there is a stage where the nuclear reticulum shows

a tendency to draw away from the nuclear membrane (Fig. 1). It is true that this effect is probably due to the fixing reagent, but the readiness with which the effect is produced both in this and the corresponding stage of the next mitosis shows that the relation of the network to the membrane—probably to the cytoplasm—is much less intimate than it is during the spirem stage.

The spirem is very coarse, the chromatin granules being unequal in size and the staining not deep. It frequently seems polarized, but at this stage no signs of splitting can be seen. The nucleolus now becomes vacuolate. Frequently, but not as regularly as in the next division, there is a large central vacuole with deeply stained inclusions. Immediately before the segmentation of the thread (Fig. 2) the nucleolus becomes swollen, irregular in form, and distinctly fibrillar in texture,—an appearance which is also seen at this stage in most of the other mitoses. Soon the nucleolar membrane disappears and the free, fluffy ends of the fibrillae project from the general mass of nucleolar substance. This is identical with what occurs in the prophase of the next division (Fig. 19). At this time the nuclear cavity has no reticulum within it.

The chromosomes now begin to appear and are often disposed in the neighbourhood of the nucleolus. The latter loses its coherence and becomes an irregular mass of granules and curved fibrils, some of them looking not unlike minute chromosomes, and, unless very carefully counter-stained, very similar in their staining reactions. Besides these, the nucleolar globule, which always accompanies the spindle, is already differentiated. This is much less deeply stained, and, except for a small vacuole which sometimes appears in it, is quite homogeneous. Two sheaves of fibres now project into the nuclear cavity, their extremities reaching about one-third of the way to the opposite pole, leaving the equatorial region free. Here are aggregated the fragmenting nucleolus and the chromosomes. The latter are considerably bigger than during the succeeding mature spindle stage; they are curved and distinctly split. Fig. 3 represents one of three oblique sections through such a nucleus. After making allowance for chromosomes that have been cut and consequently appear partly in this and partly in other sections of this nucleus, the number of chromosomes is from thirty to thirty-four.

The spindle when fully formed is intranuclear, the membrane persisting close up to the poles. The spindle proper is truncated at the poles, and in some cases (Fig. 4) is not greatly dilated at the equator. The space between the spindle and the membrane is occupied by a few mantle fibres and a large number of curved fibrils almost certainly derived from the disintegrating nucleolus. The nucleolar globule is also seen somewhere in the vicinity of the spindle—it now stains just like the cytoplasm. The chromosomes are uniformly distributed through the nuclear plate in a dense

flat disc. Those at the periphery are curved and have the free ends directed outwards. Careful countings of polar and other views give from twenty-seven to thirty-two chromosomes. The polar radiations and centrosomes are not nearly as clear as they are during the early prophase stage.

When the daughter chromosomes are halfway to the poles the nuclear membrane is still intact, and the centrosomes are visible at both poles. Between the chromatic discs and the poles the cones of fibres are clear of granules and the fibres are fine and close set, while the interzonal fibres are, as usual, fewer, far coarser, and the space between them invaded by granules and fibres. When the chromosomes reach the poles, they form two flat discs, the distal one being much greater in diameter than the basal. Very often a chromosome lags behind the others and projects towards the equator (Fig. 5). The membrane has now disappeared, so that the nucleolar fibrillae and globule are excluded from the chromatin discs (Figs. 5 and 6). The coarse connecting fibres still remain and the distal centrosome and radiations are distinct. Sometimes the basal centrosome can be made out, but there are no radiations. The lower half of the figure projects into the vacuolated region while the upper half is imbedded in the denser cytoplasm.

A membrane now surrounds each of the daughter nuclei, the diameter of which is much less than in either the preceding or succeeding stages. Instead of lying parallel in a flat disc, the chromosomes now form a tangled mass, but they are still distinct and preserve their curved form. In this condition it is often easier to estimate their number than during the late diaster stage. At this period nothing can be distinguished within the nuclear membrane besides the chromosomes. The chromosomes gradually fuse so as to form an irregular coil or reticulum with a very few meshes. This coil is very much thicker in the tetraspore mother-cell nucleus than it is in the sister nucleus. Each thick thread is at least double, the median line can frequently be seen to be lighter than the two edges (Fig. 6, upper nucleus). The strands of this irregular coil are often swollen at intervals, and there may be ten to sixteen of the swellings. Throughout this period of partial fusion the membrane of the upper nucleus, on the distal side, is very irregular, there being a marked projection towards the centrosome. A very faint reticulum now appears in the nucleus, and the condensation of the coil proceeds till very often it assumes the form of a thick open ring.

Ultimately the chromatin mass becomes spherical in form and uniformly granular or finely fibrillate in appearance, in fact it is the nucleolus. It is worthy of note that of the four mitoses described in this paper this is the only one in the telophase of which there is no differentiation of nucleolar and chromatin masses. The diameter of the nucleus is now much greater, and there is a fine but very faintly staining reticulum.

The septum dividing the stalk-cell from the sporangium proper soon makes its appearance, and the separation of the tetraspore mother-cell is completed.

THE FIRST, OR REDUCING DIVISION OF THE TETRASPORE MOTHER-CELL.

Before the initiation of the spirem the nucleus passes through a similar condition to that shown in Fig. 1, where the reticulum easily separates from the nuclear membrane. Soon, however, there is a very thin convoluted spirem distributed through the nuclear cavity. The fine granules upon this stain but feebly as yet, but here and there a few larger, but still small, deeper-stained bodies appear between the threads (Fig. 7). The nucleolus preserves for a time the fibrillar appearance already described, but as the spirem develops a vacuole appears in the former with, at first, a few smaller ones. Soon, however, there is only one fairly large vacuole which has a firm, deeply staining outline, and there are darker staining granules within it. The axis of the nucleus is once more parallel with that of the thallus, but the poles can only be distinguished with great difficulty owing to the faintness of the radiations (Fig. 7), and the centrosomes, if they exist at all, are not distinguishable. No signs were seen at this stage of the division of the centrosome, nor could the change of position of the nuclear axis be followed. The spirem gradually becomes more prominent, and shows a tendency to aggregate near the poles. A deeply staining spherical or sometimes angular body, considerably larger than any of the granules hitherto described, now makes its first appearance. It is peculiar to this mitosis, and it persists till after the splitting of the spirem (Figs. 8–12). It is always present during these stages, and there is hardly ever more than one. Whether it directly separates from the nucleolus or is formed by the fusion of the smaller granules shown in Fig. 7 could not be decided. The body will be designated for the present the *chromophilous spherule*. It should not be confused with the nucleolar globule which appears during several of the mitoses in this plant. The faintly staining cloudy nucleoplasm which appears during the synapsis is absent from this stage.

The above condition leads directly to the very interesting *knot stage* or true *synapsis*, which forms a most striking feature in the cytology of the reduction stage, but appears nowhere else in the whole history of the plant. Mottier¹ says: 'During the prophase of both divisions in the tetraspore mother-cell the behaviour of the chromatin differs strikingly from that in the higher plants. There is not developed here a regular and continuous chromatin-spirem which segments into the chromosomes, but these arise as isolated masses often differing much in size. It gives the impression

¹ 1900, l.c., p. 188.

that the quantity of chromatin is not sufficient to form a continuous spirem.' This, however, is a mistake. It would be hard to find a more distinct spirem than this, or one more interesting in its development. Many hundreds of them have been studied, and in some material they are far more numerous than spindles, showing that they persist for a considerable time. The main features of the spirem stage in *Dictyota* are so constant that no one after seeing the synapsis could possibly call it an artefact. After completing the work on *Dictyota* I began to study the cytology of *Padina*. This plant was found to agree with *Dictyota* in all the principal points, while it presented the great advantage of having the various stages sorted out as it were. In *Dictyota* the sporangia are isolated and the various stages are generally mixed up in a haphazard fashion. Thus the finding of any particular stage is a matter of chance, and in order to fill up some of the gaps several thousand sections have been examined. In *Padina*, however, the sporangia are collected together in concentric bands on the fan-shaped thallus, and in these bands many hundreds are so closely crowded together as to be in actual contact. Furthermore, all the primary sporangia in any one band are approximately in the same stage. Thus the first band behind the apical region is frequently found to have all the nuclei in the spirem stage, while the older band behind this has the sporangia in various stages of division. In very old sori, where there has been liberation of spores, new sporangia are initiated; here the uniformity above spoken of does not obtain.

The failure to recognize this stage is probably due to the fact that the tetrasporangia in Mottier's plants were too old, for it is a striking peculiarity of *Dictyota* and *Padina* that the spirem appears as soon as the mother-cell has been separated and while the sporangium is still young and small; it then disappears, and the nucleus assumes the appearance of the resting stage. In the preparation for this mitosis there are then three well-marked stages: (1) A very precocious spirem, which lasts a considerable time and goes through several interesting changes. (2) A reticulum stage, which is exactly like that of rest. It is with this that Mottier's studies commence, and his Fig. 1 comes between Figs. 14 and 15 of this series. (3) A prophase stage which is passed through somewhat quickly.

The Synapsis Stage. The spirem is now very long, thin, and deeply stained. It is closely coiled up into one or two clumps of angular loops (Figs. 8 and 9). The two knots appear closely pressed against the nuclear membrane, one opposite each centrosphere. The centrosome and radiations are still very indistinct, those shown in Fig. 8 being the best observed. The nucleus is very large, but the membrane is thin and ill-defined. The knot is so pressed against the membrane that it is often difficult to decide whether the spirem is entirely within it, and it

frequently seems as if the thread were directly continuous with, or attached to some of the cytoplasmic fibres. Very often, also, the long angular loops of the spirem seem to radiate from the vicinity of the centrosome towards the nuclear cavity. Although generally in two knots, the spirem is continuous, one or several connecting threads stretching across the cavity from one knot to the other. These frequently pass over the surface of the nucleolus, and can be seen to be in contact with it (Fig. 8). The behaviour of the latter is interesting. It invariably becomes swollen, very irregular in shape, and greatly vacuolated, *but never fibrillar*. Many cases are seen where a portion is drawn out towards the spirem, to which the apex of the projection is attached (Figs. 9, 10). Sometimes big roundish lumps appear to be on the point of separating from it, and at others the whole nucleolus seems to be fragmenting. This, however, it never does; as soon as the synapsis is over the nucleolus resumes its spherical form. Fig. 9 shows a case where there is only one knot. It sometimes happens that the spirem is entirely on the basal side of the nucleus (i. e. on the side nearest the 'basal' or 'stalk-cell') and not in the vicinity of the poles. In *Padina* it is most frequently either on the basal or on the distal side, or both. This has probably some reference to the fact that here the sporangia are compressed laterally by contact with each other, so that the axis of the first division spindle is vertical to the surface of the thallus instead of parallel to it as in *Dictyota*.

The other constituents of the nucleus at this period are the spherule above described and a small quantity of cloudy nucleoplasm, which on closer examination resolves itself into a very fine reticulum which, however, stains but slightly.

The staining reactions at this stage are as follows. The spirem, spherule, and the physodes stain deeply with the chromatin stain, the two latter often showing different shades of colour. The nucleolus, chloroplasts, and cytoplasm take the basic stain, the two former more deeply than the latter.

It is very evident that there is an intimate relation between the spirem and the cytoplasm at this stage, and that communication between them is chiefly localized at the poles. The frequent connexion between the nucleolus and the spirem, as well as the poverty of the former in chromatin, both seem to indicate that much, if not all, of the chromatin comes out of the nucleolus.

The Beaded Spirem Stage. The thread now becomes thicker, and the chromatin discs can more easily be seen (Fig. 11). The knots loosen out and the thread becomes distributed more uniformly over the membrane on the basal side of the nucleus, more rarely over the upper side. The cloudy nucleoplasm is also generally aggregated along the basal side, so that the spirem seems embedded in it. The nucleolus gradually

becomes more spherical, and a large vacuole appears in it containing minute fibrils similar to those shown in Figs. 11 and 12. After a time the spirem is more uniformly distributed over the whole surface. Fig. 11 does not show this very clearly, as the section is a median one, the rest of the spirem being in other sections. The nucleoplasm begins to diminish in amount; some of it is seen as little fluffy threads attached to the spirem.

The staining reactions are peculiar. With brazilin and picric-Hoffman-blue, for instance (the former being the chromatin and the latter the plasma stain), the spirem and the nucleolus stain reddish brown, the spherule yellowish red, while everything else, including the fibrils in the nucleolar vacuole, stain blue. Later on the spirem loses its chromatin reaction and colours blue like the chloroplasts. No definite conclusion respecting the chemistry of the nucleus can be drawn from this, but it is interesting to note how the nucleolus and the spirem reverse their respective colour reactions in the knot and the later spirem stage.

The Split Spirem. The thread now instead of lying along the nuclear membrane becomes more evenly distributed through the cavity. Longitudinal fission can be seen here and there, and before long it is found to form two distinct threads which often twist round each other (Figs. 12 and 13). The granules are irregular in size and more distant from each other. As in the preceding stage they show no special affinity for chromatin stains. The nucleolus stains more deeply, and shows a very big vacuole with inclusions. The spherule is still present, and besides the nucleole it is the only nuclear structure that colours with chromatin stains. The nucleoplasm diminishes in amount and finally disappears, being probably incorporated in the reticulum formed out of the split spirem. The centrospheres are still very indistinct, the mother-cell is bigger and more vacuolated than in the preceding stage.

The 'Resting Stage.' The next stage in the history of the spirem is difficult to make out. Instead of a comparatively small number of threads which are split, one sees a large number of very fine threads with numerous granules, very variable in size and disposition, crossing the nuclear cavity in all directions, and in many places appearing to form a reticulum (Fig. 14). Whether this appearance is due to the mere separation of the halves of the split spirem, with a lengthening and thinning out of the threads, or of this with a second split superadded, it is very difficult to make out. The halves undoubtedly do separate widely, and the identity of the constituent threads is completely lost, but a careful examination of a very large number of examples failed to disclose any clear evidence of such a second split. On this account, and after a close study of the prophase stage (Figs. 17-19), I have come to the conclusion that there is no second split at all, and that the appearance seen in Figs. 14, 15, is due to an alveolation of the separated halves of the chromatin thread

and a joining together of the very fine reticulum thus produced by fine cross threads, so that the nucleus appears to be in a state of rest.

The spherule now stains paler than before, then breaks up into two or several smaller ones, and at the close of this stage completely disappears.

After a time the sporangium presents several striking features. The cytoplasmic constituents when seen in section are arranged in evident zones, so that the term 'zonated' might be appropriately applied to it. The centrosomes and radiations are now exceedingly distinct at both poles, and there is a narrow zone of kinoplasm extending round the sides of the nucleus. This area is quite clear of chloroplasts. Immediately outside, and in sharp contrast to it, there is a zone of dense protoplasm with crowded chloroplasts which stain very deeply, generally taking more or less of the chromatin stain (Fig. 15). The outer region is vacuolated, and here the chloroplasts generally stain like the cytoplasm. This is not included in the figure.

The nuclear membrane is firm and clear. At the poles it is generally very irregular; the example selected to draw Fig. 15 from does not show this very clearly. It generally forms projections towards the centrosomes, and this causes a number of folds and wrinkles. Frequently a conical extension of the polar membrane has its apex touching the middle of the rod-like centrosome, the two ends of which are free. Occasionally the projection is truncated at the apex, and is in contact with the rod along its whole length. When this is viewed from the side the centrosome looks almost like a fold of the nuclear membrane.

In *Padina* there is the same arrangement of the cytoplasm into concentric spheres, though perhaps not quite so pronounced as in *Dictyota*. The centrosome and radiations are very much less distinct.

The nucleolus is spherical and somewhat deeply stained, but spongy, with large and small vacuoles. The only other constituent is the reticulum already described.

The Prophase Stage. Very soon thick cloudy strands appear in the nucleus. There seems to be little, if any, diminution of the reticulum, so that it is not clear whether these are derived from a further condensation of the denser strands of the reticulum or not. As the cloudy bands assume a more definite form, a longitudinal split makes itself evident in them (Fig. 16). When these bodies have still further condensed, and assumed the forms of chromosomes, they are seen to be either closed rings or looped-up bands with their free ends crossing. This is well shown in Fig. 17 from *Taonia*. The next figure (18) represents three rather thick sections of a similar nucleus from *Padina*. These include all the chromosomes, and they are uncut with the exception of the one in the extreme left of *c*, the bend joining the two halves of which is shown in section *b*.

This shows clearly that the loop-figures have not been caused by cutting off the ends of elongated rings. The failure to find evidences of a second split of the chromatin thread, and a careful study of the development of the chromosomes, inclines me strongly to Farmer and Moore's view of the origin of these bodies, as recently advanced before the Royal Society¹. According to this the space inside a ring-chromosome, or that between the two limbs of a loop-figure, does not represent the first split, but rather the space between the two constituents of a bivalent chromosome bent over on itself so that the two ends meet, or the two limbs cross, or some modification of these forms is brought about. The split itself (the only split according to this theory), though partly obliterated by the condensation of the chromosomes, is still evident in the free ends of several of the chromosomes in Figs. 17 and 18. During this stage the nuclear reticulum is somewhat dense and the nucleolus minutely vesicular.

By the time the spindle cones begin to develop the chromosomes have still further condensed until they are small and deep-staining, but the ring form is very prevalent (Fig. 19). Thus, although a long time has elapsed since the spirem stage, and in the meantime all traces of it have been lost in the apparently formless reticulum, yet the chromosomes make their appearance already segmented and longitudinally split. The length of the spirem period and the comparative shortness of the prophase irresistibly suggest that the arrangements for mitosis are completed, or nearly so, in the former; that the grouping of the various constituents is there determined, and however disguised in the reticulum of the post-spirem stage that it still exists. This is equivalent to saying that the spirem maintains its identity throughout, and that when the stimulus is given which produces the prophase there is a rapid condensation of the previously extended, unrecognizable spirem into compact, deeply stainable, split, bivalent chromosomes. This does not exclude the possibility of substance passing into the chromosomes from the nucleolus. From actual observation, however, we can determine that this must be small in amount, for the bulk of the nucleolar substance is otherwise accounted for. In Fig. 19 the nucleolar membrane has disappeared, and the contained fibrillae, as deeply staining as the chromosomes, lie together in an irregular mass, some of them with their free ends projecting. Other sections show, as in the case of the stalk-cell division, that the spherical globule subsequently seen near the spindle is also formed out of the disintegrating nucleolus.

The Spindle Stage. Of this a great many examples have been studied, and they all agree in their main features. The spindle is intranuclear, very narrow, and nearly iso-diametric. The nuclear membrane is intact excepting at the poles, where there are evident gaps nearly as

¹ Proc. Roy. Soc., vol. lxxii, p. 104, 1903.

wide as the spindle itself (Fig. 20). There are divergent mantle-fibres between the spindle and the membrane—these rarely reach as far as the equator. The radiations and centrosomes are much fainter than during the post-spirem stage. Fig. 22 shows a very good centrosome from an oblique section; there is a very similar one in the section containing the other pole.

In many of the figures the spindle-fibres seem tense and nearly straight, while the mantle-fibres are lax and wavy (Fig. 20). The former give one the impression of having contracted and shortened till in some cases (not so well shown in the one drawn) the polar edges of the membrane are curved inwards and the centrosome is sunk within the gap. Here, as in the preceding mitosis, there are nucleolar fibrillae, which, as observed by Mottier, often stain almost as deeply as the chromosomes, but when a deep counterstain has been employed there is no difficulty in differentiating them from the chromatin. The nucleolar globule is nearly always present, generally at the periphery in the equatorial region. It always stains like the cytoplasm.

The chromosomes, as already stated by Mottier, are sixteen in number, and decidedly heterotype in character. Figs. 20 and 21 show their form, and it seems hardly necessary to describe them in greater detail. Their small size makes it difficult to decide how they are placed on the spindle; and whether the division is such as to bring about true reduction or not is for the same reason equally difficult to decide.

In sections parallel to the surface of the thallus it happens occasionally that the nuclear figure is flattened so as to widen out considerably at the equator. In these cases the chromosomes are widely separated, and consequently more easily counted.

The Anaphase Stage presents no peculiar features—the description of the stalk-cell division anaphase would almost apply to this. Mottier, however, states that chromosomes on their way to the poles often fuse together into larger masses. I have seen no instances of this in healthy cells. From the post-spirem stage onwards a large number of tetrasporangia become arrested in their development, and frequently this takes place while the nucleus is in mitosis. In such cases the chromatin shows various abnormalities of structure. As a rule, however, one finds it easy to recognize such abnormal examples from the peculiar appearance of the cytoplasm. Occasionally, in the earlier stages of degeneration, there may be difficulty in deciding whether a phenomenon is normal or not. Here one has to depend entirely upon comparison of a large number of cases; from such a comparison I have come to the conclusion that the fusion of chromosomes above spoken of never takes place excepting in degenerating sporangia.

The Telophase Stage is strikingly different from that of the stalk-cell

division, and though more like that of the first segmentation of the spore there is a difference in the condition of the chromatin mass. Mottier has good figures of this stage (see his Figs. 8, 9). Instead of the chromosomes forming one large, deeply stained body, two, sometimes more, are formed. One is very large, irregular in form, and composed of fine fibrillae and granules, while the other is smaller, denser, and more suggestive of a nucleolus (Fig. 23). The former is undoubtedly the chromatin. In this condition it is exactly like a stage in the fragmentation of the nucleolus. A little later the chromatin fibrillae are seen to be more widely distributed through the nucleus (Fig. 24); eventually they disappear altogether. Coincidentally with this the reticulum becomes more and more evident and the nucleus increases in size (Fig. 25).

THE SECOND MITOSIS IN THE TETRASPORE MOTHER-CELL.

The axes of the two daughter-nuclei are now parallel to each other and to the surface of the thallus, but at right angles to that of the stalk-cell. Thus a section tangential to the thallus shows both nuclei longitudinally. In such a section a first division spindle would also be cut longitudinally but it would be parallel to the axis of the stalk-cell, whereas in the stalk-cell division the figure would be cut across so as to give a polar view. During the prophase stage the chloroplasts are densely aggregated in the vicinity of the two nuclei, while the median plane is sharply marked out by their absence or scarcity. The two nuclei are somewhat flattened on the outer surfaces, whence radiations curve round the ends of each nucleus towards those of the sister nucleus. The spirem is thick and irregular, and it soon segments, the nucleolus at the same time going through the usual process of disintegration into fibrillae (Fig. 26). In both nuclei 'kinoplasmic' activity is confined to the nuclear surfaces remote from each other—those nearest the periphery of the mother-cell. It follows from this that in the early spindle the cones of fibres are not in a straight line. This has been well shown by Mottier. In a nucleus similar to his Fig. 12 the scattered chromosomes have been counted without difficulty, and here again the reduced number has been found to obtain. The mature spindle (Fig. 27) is an interesting object, especially when viewed in profile. Owing to the apparent 'kinoplasmic' repulsion above described the spindle is placed against the outer side of the nucleus, the sister nucleus on the opposite side of the cell presenting the same features but reversed as to direction. Even at maturity there is frequently a slight curve in the figure. The centrosomes are not easy to make out, but the radiations are clear, and as before curved towards those of the sister nucleus. The spindle itself is elongated and narrow, the chromosomes are curved, and countings of both oblique and polar views leave no doubt that the number is the reduced one. The remaining constituents—

mantle-fibres, nucleolar globule, and fibrils—are shown in Fig. 27 and call for no special comment. Mottier says that 'the invariable tendency of the chromosomes to collect and fuse into larger masses has made it impossible to determine accurately their number in this division. In the equatorial plate often only two large chromatin masses are seen (Figs. 14, 15).' This description does not apply to the material examined by me, where the chromosomes remain distinct up to a late stage in metakinesis. Both in the prophase and spindle stages the chromosomes are clearly seen to be homotype in character.

The diaster stage is of the usual type. The figure becomes very narrow and elongated as a rule (Fig. 28). The membrane often persists until the daughter chromosomes are nearly at the poles. (Mottier says it 'disappears soon after the spindle is mature, generally before metakinesis.') The pale-staining nucleolar globule is still visible at this stage.

The formation of the daughter-nuclei shows the same phenomena as in the preceding division. Here, however, the nuclei are exceedingly small. The radiations completely disappear. In *Padina* the four spore-nuclei within the sporangium are bigger than those of *Dictyota*, their chromatin thread is prominent during the resting period, and the cytoplasm is differentiated into distinct concentric spheres.

KARYOKINESIS IN THE GERMINATING TETRASPORE.

The tetraspore on being liberated, instead of assuming a spherical form as is the case with the oosphere, is nearly always elongated and almost cylindrical. The position of the nucleus is indicated by a lighter area. Division of the nucleus takes place in twelve to sixteen hours. The resting nucleus is much larger than it was in the tetrasporangium. The condition of the cytoplasm—the greater width of the meshes and the separation of the plastids—shows that this is due more to distension consequent on liberation from the confined space of the tetrasporangium than to growth. One consequence of this is that the parts of the mitotic figure are much clearer here than in the sporangium.

Before the development of the spirem the usual reticular structure is shown. At the same time the nucleolus has the fibrillar structure so frequently referred to already. The spirem (Fig. 30) is coarse and unevenly beaded like that of the stalk-cell division: in this case, however, it is accompanied by a considerable amount of fine reticulum.

The spindle (Fig. 31) is intranuclear like the preceding ones. It is, however, much broader at the equator and the centrosomes and radiations are more prominent, a result probably of diminished pressure in the cytoplasm. It is a rather striking fact that no nuclear globule has been observed at any stage of this mitosis.

The chromosomes have been counted in a great many figures, and they

are always about sixteen in number. Their form is curved just like those of the stalk-cell division. During the anaphase stage the membrane persists up to a very late period (Fig. 32). The mantle-fibres, centrosomes, and radiations are very distinct; in fact all the accessory structures are far more easily seen here than in the preceding mitoses.

The curved chromosomes aggregate at the poles in the usual way with their free ends towards the equator; a membrane is formed; then, as in the stalk-cell division, they lose their polarity but remain distinct enough to be countable for some time longer. Here (Fig. 33) again it is seen that the number is the reduced one. The centrosomes and radiations, together with the connecting fibres, are still clear. A striking feature of this and of the next stage (Fig. 35) is the projection of the nuclear membrane towards the centrosome, already seen in some previous stages.

Fig. 34 shows the chromosomes to be accompanied by a distinctively staining body which in all probability is the rudiment of the nucleolus.

The fusion of the chromosomes goes on at first somewhat like that of the stalk-cell division. Here, however, two masses are formed (Fig. 35), of which one is larger and more irregular in form than the other. A reticulum also appears which at first stains but faintly. Later on the smaller of the two masses dwindles in size and finally disappears, the reticulum at the same time becoming more deeply stained and the other body (the nucleolus) assuming a spherical form.

ABNORMALITIES.

There are various abnormal developments here which it might be profitable to study in greater detail:—

(1) Undivided tetraspore mother-cells are sometimes liberated, and several instances have been observed of their nuclei in the prophase or even in the spindle stage. Most of these, however, were evidently arrested, and there is as yet no evidence to show whether karyokinesis was initiated before or after separation from the thallus.

(2) Several instances have been seen of liberated sporangia containing two spore-like bodies, each, however, being invested with a cell-wall. In one case the nuclei seemed to be in second division prophase, with spirem and fibrillar nucleolus. In the other the late anaphase stage was seen but the chromosomes could not be counted.

(3) This is an abnormality which is of greater interest, as it occurs more frequently and may possibly be of some utility to the plant.

Towards the close of the season, instead of dividing in the usual manner to form tetraspores, the mother-cell, without greatly increasing in size or taking on a deeper colour, divides into two and then into a small mass of parenchyma. Richards¹ observed this phenomenon in *D. ciliata*, but says:

¹ Proc. Amer. Acad., 1890, p. 83.

'The significance of this multiple division could not be explained with only alcohol material; it was too frequent, at least in the specimens I examined, to be an accident.'

The parenchymatous mass may later on develop an apical cell and grow out into an elongated germling-like branch, and, as will be shown in another paper, it may remain alive when the rest of the thallus has decayed.

The cytology of this stage has not been fully worked out, but what probably happens is that the conditions (temperature and light) being unfavourable to the development of the reduction stage (it is evidently very sensitive to external influence) the stalk-cell division is followed by ordinary vegetative divisions. As far as my observation goes this mode of cell-multiplication never follows the reduction division, and when this division is abnormal the death and disintegration of the cell inevitably ensues.

(4) In *Padina* 'twin' tetrasporangia are of very frequent occurrence. After separation from the stalk-cell the mother-cell nucleus instead of going into synapsis divides vegetatively, there is a longitudinal division of the cell, then the two nuclei go through the various phases of the reduction stage, but they lag somewhat behind the neighbouring sporangia, as if they had lost time by the extra division.

CONCLUSIONS.

1. Alternation of Generations.

It has now been shown that in the thallus- and in the stalk-cell divisions of the tetraspore plant the nucleus has about thirty-two chromosomes. The first division of the resulting mother-cell is different from all the others in its long preparation for division, its elaborate and distinctive spirem stages, the heterotype character of its chromosomes, and in the fact that here the number is reduced to sixteen. The succeeding division has the same number of chromosomes, and in the young plant produced from the tetraspore the earlier mitoses all show the reduced number.

As will be shown in detail in a succeeding paper the thallus-cells of both male and female plants are probably characterized by the reduced number: it is difficult to be absolutely certain where the nuclei are so small. In the oogonial and antheridial divisions, however, there is no doubt about the number. Furthermore, in all these various mitoses there is not one that resembles the reducing division in its distinctive characters.

In the interesting series of abnormal figures observed in the parthenogenesis of unfertilized eggs the chromosomes are always scattered, and consequently easily counted, and the number is invariably sixteen, whereas the fertilized oosphere in all its segmentations shows the full number. It

it difficult in the face of these facts to resist the conclusion that the germ-ling produced from the tetraspore, with its sixteen chromosomes, is a young male or female plant, and that the segmenting oospore with thirty-two chromosomes to its nucleus is a young tetraspore plant.

There is a difficulty which will have to be explained. In certain localities experience has shown that it is most difficult to find a sexual plant. There are extensive tracts along the coast where all the plants seem to be the form *intricata*, many of which have no reproductive cells of any kind, and the remainder are nearly always tetrasporic. *Taonia* has a similar reduction division to *Dictyota*, and from analogy one would expect to find alternation here. Since the year 1897, however, I have not succeeded in finding a single sexual plant, whereas the others are found without difficulty. This of course is merely negative evidence, and after all there may be a few individuals of the gametophyte generation even in these localities. Assuming that the above observation is correct, one has to account for two things—the failure of the tetraspores to produce sexual plants, and the perpetuation of the former in the absence of the latter. This cannot be fully discussed without going into the whole question of environment, which will be done in a later paper. It may, however, be suggested:—

1. That the same unfavourable conditions that give rise to the *intricata* form also account for the absence of sexual plants.

2. That one of the several modes of vegetative reproduction occasionally resorted to by the plant, including perhaps the curious abnormal development of the tetrasporangium-rudiment above described, may enable the asexual generation to perpetuate itself indefinitely.

Some hundreds of culture experiments have been tried in order to test the validity of the conclusions arrived at from cytological evidence. Plants reared from tetraspores and others from fertilized eggs have been kept alive for months, but owing to their sensitiveness to external conditions it has hitherto been found impossible to get them to develop reproductive cells. Improved methods are being tried at present, and it is hoped that these will be successful.

It may possibly be urged that the two generations in the higher plants are generally quite dissimilar in form and structure, whereas in this case they are identical in both respects. Where, however, the two generations, as in this case, are both strictly aquatic, there seems to be no inherent necessity for a difference of form or of structure.

II. The Nucleolus.

With regard to the nucleolus, it has been seen that in the earliest stage in all the mitoses this body is always fibrillar or granular. It is a suggestive fact that the same appearance is resumed during the prophase stage,

when it swells, becomes angular, and finally its membrane disappears and the fibrillae are allowed to disperse through the nuclear cavity.

The stages of the 'reduction' nucleolus are the following:—

1. The nucleolus is uniformly fibrillar, and probably contains the bulk of the chromatin.
2. It becomes vacuolate and the spherule forms.
3. It is much distorted and attached to the spirem; the staining power becomes gradually less.
4. A large vacuole appears, with fibrillar inclusions.
5. It becomes spongy and more deeply stained.
6. It swells, becomes angular and fibrillar.
7. It disintegrates into fibrillae and a nucleolar globule, which are both excluded from the daughter-nuclei.

The distorted nucleus accompanying the 'knotted' spirem, and its frequent actual attachment to the thread, irresistibly suggest that the nucleolus nourishes the spirem at this stage. This view is strengthened by the fact that about this time the nucleolus becomes less responsive to chromatin stains.

In the succeeding stage the very large vacuole occupying the greater part of the diameter seems to show that the nucleolus has been deprived of much of its substance. The significance of the included fibrils is not clear.

The spherule accompanying the reduction spirem is most probably a derivative of the nucleolus. It stains intensely with most chromatin stains, but shows some distinctive reactions, especially its retention of Carbol Fuchsin. Very often the spherule remains bright red when the colour has been extracted from all the other constituents. It is not homogeneous—very frequently deeper stained bodies can be seen within it. It shows no definite relation to other nuclear structures and its rôle cannot at present be explained.

The disintegration stage shows that the nucleolus contains two very different substances, the fibrillar chromatin-like constituent, and the globule—plasma-like in its colour reactions. If the former substance be not chromatin, then a third constituent must be added, for it is exceedingly probable that part of the chromatin is stored up in the nucleolus previous to karyokinesis.

It was shown that in the reconstruction of the daughter-nuclei the process in the stalk-cell division is different from that in all the others. In the tetrasporangium nucleus all the chromatin is at first stored up in the nucleolus, and there is no second body within it. This may be part of the elaborate preparation for the reduction of the chromosomes. In the telophase of the next division the chromatin is aggregated in a separate mass of fibrils which, however, become dispersed through the nuclear space and

then disappear. The study of these makes it clear that in the karyokinetic stage the bulk of the nucleolus is cast out into the cytoplasm in the form of fibrillae and globule, and it does not seem that either substance is used in the formation of the spindle, for they coexist with the mature spindle up to a late stage in metakinesis. The appearance of the new nucleolus follows so quickly upon the stage when the nuclear membrane encloses nothing but the dispirem, that we are forced to the conclusion that the substance separates from the chromosomes.

The centrosomes and other accessory structures will be discussed when the cytology of the sexual generation has been described.

With regard to the development of the chromosomes it is clear that if the description given above be correct, whatever the details of the metaphase stage may be, a transverse division of the chromosomes must occur somewhere. No other theory seems to account so satisfactorily for the facts as that of Farmer and Moore, already referred to; and I regard the figures of the prophase stage in the three Dictyotaceous genera examined as strongly confirmatory of the hypothesis.

EXPLANATION OF FIGURES IN PLATES IX AND X.

Illustrating Mr. Lloyd Williams' Studies in the Dictyotaceae.

All the figures have been drawn with the aid of the camera lucida and the apochromatic 4.0 mm., aperture 1.30 of Zeiss with ocular 6 ($\times 800$). For convenience of comparison the same scale of magnification has been kept throughout, but a few of the figures having been drawn from tangential sections are apparently smaller than they ought to have been. Figures 2–19 have the stalk-cell on the lower side, and the terms 'basal' and 'distal' are used with reference to this cell.

Stalk-Cell Division.

Fig. 1. Tetrasporangium rudiment before stalk-cell division.

Fig. 2. Prophase of the stalk-cell division. The coarse spirem is beginning to segment and the nucleus is swollen and irregular in form.

Fig. 3. Early spindle formation. One of three sections, slightly oblique, showing a number of curved, split chromosomes, the fragmenting nucleolus with its globule, and the basal pole with a few spindle-fibres.

Fig. 4. Equatorial plate stage. The spindle is intranuclear. The remaining chromosomes are in another section. There is nucleolar globule on the spindle, and nucleolar fibrils between it and the membrane.

Fig. 5. Anaphase stage. The two centrosomes are visible, but radiations are confined to the distal pole. The nucleolar globule is on the connecting fibres.

Fig. 6. Telophase stage. The chromosomes form an irregular coil the strands of which in places appear double. The nucleolar globule is seen to the right. The tetraspore mother-cell nucleus is bigger than that of the stalk-cell, and its membrane is drawn out towards the distal centrosome.

The First or Reduction Division in the Tetraspore Mother-Cell.

Fig. 7. Early spirem. The nucleolus is vacuolated, and there are a number of small, deeply staining granules which probably fuse together to form the 'chromophilous spherule' peculiar to this mitosis. The radiations, to the right and left, are but faintly suggested, and no centrosomes can be distinguished.

Fig. 8. The 'knot' or 'Synapsis' stage. The spirem is in two knots near the very faint centrospheres. The spherule is seen to the left.

Fig. 9. Another example, showing the distortion of the nucleolus and the intimate connexion between it and the spirem.

Fig. 10. A slightly later stage. The spirem is spread out on the basal side. A strand is shown attached to the nucleolus. The axis of the nucleus is not median but nearer the basal side.

Fig. 11. The spirem is more evenly distributed over the membrane, and is distinctly beaded, with fine fluffy threads projecting laterally. The nucleolus has a large vacuole with included fibrils and granules.

Fig. 12. The spirem begins to split in two, and the staining is less deep. The section is transverse to the nuclear axis.

Fig. 13. A later stage in the splitting. The nucleolus and spherule appear in one of the other four sections through the nucleus. The reticulum becomes fainter.

Fig. 14. The identity of the chromatin thread has been lost, and the nucleus appears as if in a state of rest. The spherule is still present.

Fig. 15. A later stage. There are denser strands in the reticulum, the spherule has disappeared, the membrane, centrosomes, and radiations are more distinct, and the cytoplasm is differentiated into separate zones.

Fig. 16. Early prophase. Thick cloudy bands appear, with indications of longitudinal splitting. The section is not median, and consequently does not include the nucleolus and centrosomes.

Fig. 17. (*Taonia*). The chromosomes appear as loops with their limbs crossed, or as closed rings. The free ends of the chromosomes frequently appear split. The nucleolus is minutely vesicular.

Fig. 18, *a, b, c* (*Padina*). Three sections of the same nucleus, showing the reduced number of chromosomes, all of which except one are uncut.

Fig. 19. The chromosomes reduced in size and ready for being placed on the spindle. A few spindle-fibres have already appeared, and the nucleolus is breaking up into fibrillae.

Fig. 20. Intranuclear spindle with mantle-fibres and nucleolar fibrillae. (The globule is in another section.) The chromosomes are heterotype in character.

Fig. 21. A slightly oblique section, showing more clearly the form of the chromosomes. The number is sixteen, but those in a lower focus have not been drawn.

Fig. 22. One of the poles of the preceding figure, showing the curved rod-like centrosome. The section containing the other pole is very similar.

Fig. 23. One of the two daughter-nuclei of the first division. There is a large mass of fibrillar chromatin and a small nucleolus.

Fig. 24. A later stage. The chromatin becomes distributed through the nucleus and the nucleolus increases in size.

Second Division in the Tetraspore Mother-Cell.

Fig. 25. Early prophase. Nucleolus swollen, irregular, and fibrillar. The section is transverse to the nuclear axis.

Fig. 26. A number of short curved chromosomes have appeared, and the nucleolus is irregular and fibrillar.

Fig. 27. Spindle. The polar radiations are directed towards those of the sister-nucleus, the membrane on the inner side is intact, the spindle being on the opposite side. A nucleolar globule is present.

Fig. 28. Anaphase stage. The nuclear cavity is much narrower and curved. The chromosomes though small show no signs of fusion.

Fig. 29. One of the four nuclei of the sporangium before spore differentiation.

The First Division in the Germinating Tetraspore.

Fig. 30. Prophase. Very coarse spirem and fibrillar nucleolus.

Fig. 31. Spindle with curved chromosomes (one has lagged behind). There are two sections of this, and the number of chromosomes in the two together is sixteen. The radiations are distinct; there is no globule.

Fig. 32. Anaphase stage. Membrane still intact at the sides.

Fig. 33. 'Dispirem' stage. The nucleolar membrane projects towards the very distinct centrosome.

Fig. 34. Polar view of daughter-nuclei before fusion of chromosomes. About sixteen may be counted. Near the bottom of the figure a small body staining differently to the chromosomes is seen. This is probably the beginning of the nucleolus.

Fig. 35. A later stage than Fig. 26, where a chromatin mass, a nucleolus, and a reticulum have been differentiated in each daughter-nucleus.

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19.

Williams, J. Lloyd. 1904. "Studies in the Dictyotaceae. I. The Cytology of the tetrasporangium and the germinating tetraspore." *Annals of botany* 18, 141–160. <https://doi.org/10.1093/oxfordjournals.aob.a088948>.

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