Nuclear Division in *Tetraspora lubrica*.

BY

F. McALLISTER.

School of Botany, University of Texas.

With Plate LVI.

IT is principally in those forms of animals and plants in which the nuclei are extremely minute, thus rendering the interpretation of the data uncertain—and in unicellular organisms or in those forms in which the cells are so isolated that great difficulty is experienced in finding numerous stages of nuclear division,—that mitosis is described as departing radically from the firmly established series of changes described for the higher organisms. Probably more different types of nuclear division have been reported for the protozoan cell than all others put together.

To cite a few of the variations in mitosis reported for the protozoan cell—the nuclear material may not be collected in a nucleus, but is distributed throughout the protoplasm of the cell (Bütschli's 'distributed nucleus'), as is the case in *Tetramites* according to Calkins (4), and in *Tracheloceroa* according to Gruber (20); or the chromatic material may be collected in a nucleus but does not aggregate to form chromosomes—a fission of the chromatic granules occurring instead of a fission of chromosomes, as is the case in *Euglena*, according to Keuten (28) and others. In *Noctiluca*, according to Ishikawa (23, 24) and Calkins (5), chromatic bodies aggregate to form chromosomes.

The origin of the spindle or its equivalent is also very variable according to the published accounts. A definite intranuclear spindle, with centrosphere-like bodies at the poles, is described for *Euglypha* (Shewiakoff, 43) as well as for other forms. In *Euglena*, according to Keuten (28), Dangeard (11), and others, an intranuclear body, the 'nucleo-centrosome', is made responsible for the division of the nucleus. This persistent body divides and the resulting halves move apart, though connected by an isthmus of the same material. The chromatic material groups about the two halves, and as they move apart the chromatin bodies pass to the poles with the 'nucleo-centrosome', and there organize new nuclei with the persistent body in the centre.

In *Acanthocystis*, according to Schaudinn (12), the centrosphere lies in the cytoplasm. It divides and its halves separate, moving to the poles of the nucleus. There seems little essential difference between the mitosis here and that in the other animal mitoses, in which asters are involved. In *Paramoeba*, according to the same author, the only important difference lies in the difference in the size of the centrospheres 'Nebenkörper', which are nearly as large as the nucleus. Wilson (54) says: '*Paramoeba* appears to differ from *Euglena* mainly in the fact that at the close of division the sphere is in the former left outside the daughter nucleus, and in the latter enclosed within it.' There seems, however, a greater difference than this, for in *Euglena* no spindle is described or figured, while in the case of *Paramoeba* a very definite spindle figure is reported, with the large centrospheres at the poles.

In the light of these divergent types of nuclear division in the Protozoa, mitosis in the Protophytes, and especially in those forms regarded as most closely related to the Protozoans, is of especial interest. Thus far no results have been obtained in any of the green plants which seem in any way to correspond with the results reported by investigators on the Protozoa.

Mitosis in *Spirogyra* has been the subject of numerous investigations—probably more than in all other Algae combined—but very great variations exist in the accounts of mitosis in this Alga, and the discrepancies are so marked as to arouse the suspicion that the described appearances cannot all be normal. If we accept the reported accounts of mitosis in *Spirogyra* as accurate, we have in this genus greater and more fundamental variations in the phenomena of nuclear division than have been reported in all the remaining green plants from the Algae to the Angiosperms. The chromosomes have been reported as arising entirely from the nucleole (33, 34, 2) or partly from the nucleole and partly from the reticulum (15, 16, 56), or entirely from the reticulum (50, 46). Lutman's work on *Closterium* (31) seems to prove clearly that in this Conjugate nuclear division follows the well-known steps established for the higher plants. Van Wisselingh (57), working independently, came to the same conclusions at about the same time.

In view of the great similarity in the most conspicuous and constant characteristics in the nuclear division in all the green plants outside of *Spirogyra* and the other investigated Conjugatae, Moll's (36) suggestion that we should not expect the same mitotic phenomena in all species of *Spirogyra* must be regarded as based upon an unstable foundation.

The literature on the mitosis in *Spirogyra* has been recently fully reviewed by Berghs (2), Lutman (31), and others, so that a detailed review here seems superfluous.

Strasburger's earlier work on *Cladophora* (45) has recently been substantiated and extended by Němec (37). According to these accounts, the

chromosomes—about thirty in number—arise from the reticulum independently of the nucleole. A distinct bipolar spindle, with no traces of a centrosome or centrosphere, appears at metaphase. In one respect only—the persistence of the nucleole—is there any divergence from typical division as known in the higher plants. The nucleole, according to Némec, becomes much elongated, the two resulting parts remaining connected by a slender strand of nucleolar material. Not until the daughter nuclei are partly formed does this connecting strand disappear. The main elements of the nucleus, as well as of the method of nuclear division, are the same in this Alga as in the higher plants.

Tuttle (50) has recently confirmed the earlier work of Strasburger (45), Wille (52), and Mitzkewitsch (35) on nuclear division in *Oedogonium*, and has published a fuller account of mitosis in this Alga than any one of these investigators. His results clearly show the origin of a spireme thread from a reticulum, independently of the nucleole,—the formation of the chromosomes from this spireme, and the splitting of these and the distribution of the split halves to the daughter nuclei, where they become reconverted into the reticulum of the new nucleus. The nucleole forms no morphological part of the chromatic thread or the chromosomes. It is interesting to note that here the spindle is intranuclear.

Timberlake's work (48) on *Hydrodictyon* dealt with very minute nuclei and a small number of phases of division. Nevertheless, as he says, 'enough stages stand out sharply to show that the process is essentially the same as in the higher organisms.' A spireme is formed from the reticulum. This segments to form about ten chromosomes. These pass into the equatorial plate stage. Two groups of chromosomes are formed, probably by the splitting of each of the ten original chromosomes, although this splitting was not observed because of their very small size. Centrosome-like bodies are described and figured at the poles of the spindle figure.

Yamanouchi (59) has recently published a short note upon what he regards as a new species of *Hydrodictyon* from South Africa. A brief reference is made to nuclear division. He is of the opinion that the spindle is intranuclear and has centrosomes,—but his few small, diagrammatic text-figures certainly do not go far toward establishing the presence of centrosomes for this species. He refers to numerous chromatophores which 'have two functions, one to produce characteristic pyrenoids and the other to form reserve starch grains'. Starch formation was not observed in connexion with pyrenoids but it is formed, perhaps by secretion, in the plastids near one margin. If this brief reference is substantiated by more exhaustive investigation, we have in this Alga, plastids which cause starch deposition in a manner apparently identical with that in the seed plants,—the pyrenoid not functioning as a starch-forming cell organ.

Allen's research on *Coleochaete* (1) has shown that the reduction

divisions are here not essentially different from those in higher forms. In early prophase, apparently as the reticulum passes into a spireme, the chromatic material aggregates at one side of the nuclear cavity to form the synaptic knot. As it emerges from synapsis the chromatic material is in the form of a spireme thread. The nucleole retains its identity until about the time of the segmentation of the spireme to form the chromosomes. The further stages of mitosis are also in no essential way dissimilar to those of the higher plants.

Dangeard's work (9, 10) on certain of the Chlamydomonadaceae, while in no way complete or exhaustive in respect to any single species, has nevertheless shown that in this interesting group, regarded by many algologists as having the closest relationship with the Protozoans and even regarded as Protozoans by most zoologists, nuclear division does not vary essentially from that as established for the higher plants.

Working on *Chlamydomonas*, *Phacotus*, *Chlorogonium*, *Carteria*, and *Polytoma* he has described and figured phases of the division of the nucleus in which a definite number of chromosomes arise from a reticulum. The spindle arises from the cytoplasm and lacks centrosomes. There is no 'nucleo-centrosome' or equivalent body such as is reported by the same author, as well as others, for *Euglena*. Dangeard is of the opinion that the type of nuclear division in the Chlamydomonadaceae ('teleomitose') is of a higher type of development than that as determined for the Euglenidae ('haplomitose'),—and he proposes this difference in nuclear division as a character by which to separate the former group from the latter.

Davis in a discussion of spore formation in *Derbesia* (13) has given a few nuclear figures from the germinating spores. According to this brief reference, the spindle is intranuclear with minute granules at times to be seen at the poles. The spireme arises independently of the nucleole.

Fairchild (17) is inclined to believe that in *Valonia*, 'bei einigen Kernen', centrosomes with faintly defined asters are to be identified. He says, however,—'ich bin nicht sicher, ob mir wirklich Centrosomen vorlagen oder nur die convergirenden Enden der Spindelfasern, weil bei den ruhenden Kernen keine solchen Punkte zu finden waren.' The spireme arises from the reticulum and the nucleole contributes no morphological elements to it.

Debski (14) has shown that the nuclear division in *Chara* is more nearly similar in all its phases to mitosis in the higher plants than in any other Alga. The mode of spireme formation, the formation of the spindle and the cell plate seem almost identical with these processes in the nuclei of the higher plants.

Golenkin (19) believes, from evidence obtained from *Hydrodictyon* and *Sphaeroplea*, that in the resting condition of the nuclei of these Algae the chromatin is accumulated in the nucleole. He regards nuclei of this type as primitive, probably occurring in the lower green Algae.

It is of interest here to note that according to the account of Lauterborn (29) the nuclear division in the diatom *Surirella*,—unlike that of any other known plant,—has much in common with the type of mitosis that is reported for *Acanthocystis*, *Paramoeba* and other forms. According to Lauterborn's account, a central spindle is formed from a 'centrosome' lying outside the nucleus. The chromatin forms a spireme which breaks up into long chromosomes. The chromosomes become arranged about the cylindrical central spindle in the form of a ring. The ring-like mass separates into two groups which move to the poles where they organize the daughter nuclei.

The general details as to the formation of the central spindle may be said to be confirmed by Karsten (25) in his recent work on the reduction division in *Surirella saxonica*. Karsten shows clearly that here the spireme thread is organized from the reticulum independently of the nucleole. The relationship of this peculiar group to the Chlorophyceae is undoubtedly very remote.

Though differing somewhat as to the exact location of *Tetraspora* in the classification of the Chlorophyceae, nearly all students of the Algae seem agreed that the relationship of this group to the Chlamydomonadaceae is very close. There seems to be similar agreement in regarding the latter group as being the lowest of the green plants. Gay (18), Wille (53), Oltmanns (39), Chodat (7), and West (51) are practically in accord in placing the Volvocaceae as the lowest family in the order Protococcales with the Tetrasporaceae (or Palmellaceae) as the family next in order. Collins (8) places the Tetrasporaceae between the motile unicellular forms and the motile colonial forms.

Since Reinke's account (41) of the life history of *Tetraspora* in 1878 practically no work has been done upon this interesting genus. Brief references by Gay (18), Chodat (6) and West (51) seem to confirm, however, the details of this life history. Biciliate zoospores are reported and smaller biciliate isogametes. The latter fuse to form a zygote which,—according to Reinke, retains the cilia of the two gametes and swims about for a time evidently entering into a resting stage at a later period. Only very meagre references are made to the details of the structure of the cell.

The *Tetraspora* species which has been used in this study is common in the vicinity of Ithaca, N.Y., growing in shallow running water attached to the rocky bed of the stream. The lamellate, gelatinous colonies are usually buoyed up by entangled bubbles of gas. The length of the gelatinous masses varies from ten to forty millimetres, the diameter being from four to ten millimetres. The cells of the colony are approximately spherical and vary in diameter from seven to thirteen microns, depending of course upon the amount of growth which has taken place since the last division. The material agrees with the description of *Tetraspora lubrica* in many respects, though the characterization is not entirely satisfactory. All three

species given by Collins (8) overlap in regard to the size of the cells,—the specific characters being based to quite an extent upon the form of the thallus.

Colonies brought into the laboratory during afternoons in July and August and kept in small glass dishes were quite sure to form abundant zoospores upon the following morning. If the morning were cloudy the period of maximum zoospore formation might be as late as eleven o'clock. During the afternoon they were produced in much smaller numbers. Often a second crop is produced the second morning and some may even be formed on the third and fourth days.

In many instances, on the second and third mornings after the fresh material has been brought in, the cells in certain areas of the thallus are seen to be in active division. Eight cells are usually formed from one vegetative cell,—though at times but four are formed. These latter cases are probably from the smaller vegetative cells. This cell division goes on rapidly, and in a short time much of the gelatinous thallus has disintegrated.

The new cells formed by this rapid division become biciliated and soon swim away. Later they may be seen in various stages of fusion in pairs. I found no evidence, however, of their retaining their motility after fusion. Neither was there evidence of the formation of thick-walled resting spores.

During this period of rapid nuclear and cell division, leading up to the formation of gametes, many mitotic figures are easily obtained.

The chlorophyll of the cells of *Tetraspora* occupies a cup-shaped area at one side of the spherical cell. A relatively large flattened pyrenoid lies in the thickened base of the cup (Pl. LVI, Fig. 1). This distribution of the chlorophyll can easily be determined in living cells, but in material fixed in Flemming's or Merkel's fixative and stained in Flemming's triple stain or in Heidenhain's iron haematoxylin stain, no difference can be observed in the texture or staining reaction of the protoplasm of the chlorophyll-bearing area and that of the protoplasm surrounding the resting nucleus.

The resting nucleus of *Tetraspora* conforms in its general details of structure with the better known nuclei of the vascular plants. By suitable staining with Flemming's triple stain or with Heidenhain's iron-alum haematoxylin, a very delicate reticulum with numerous net knots can in all cases be demonstrated (Figs. 2, 3 and 26). The reticulum is so delicate, however, in these minute nuclei that with careless staining it may not be visible at all. In such instances the nucleole seems to lie in a clear area which usually shows no evidence even of a nuclear membrane. I have had a similar experience in staining the nuclei of *Ulothrix zonata*. These results are suggestive and may indicate that in those cases in which the nuclei of Fungi and Algae which are cited as lacking a reticulum and having all the chromatin collected in the nucleole,—the staining has been at fault. This

may possibly be the explanation of Golenkin's results with *Hydrodictyon* and *Sphaeroplea*.

In the early prophases the net knots become more conspicuous,—increasing in size until the chromatic granules are conspicuous blue-staining bodies many times the size of the original knots, and apparently of uniform size. Accompanying this increase in size there is an apparent decrease in the number of the net knots. Figs. 4 and 6 show stages in the development of these bodies. Notwithstanding these marked changes in the nuclear contents the reticulate condition persists in nuclei which have relatively large chromatin bodies (Fig. 5).

The nucleole apparently remains unchanged during the period of increase in chromatic material. Fig. 6 shows a nucleus in which the chromatic material is nearly at the stage of spireme formation while the nucleole is still intact. It seems perfectly clear that the nucleole does not here undergo disintegration during the increase of stainable material in the nuclear cavity. It does not disappear until the spireme material is all formed,—therefore any participation by the nucleole in the formation of the spireme thread must be very slight indeed.

The uniform chromatic bodies of this much modified reticulum apparently become arranged side by side or in a row to form the spireme thread, which, although at first appearing to be of irregular diameter, does not seem to be made up of definite 'chromomeres'. If this spireme is ever in a uniformly distributed condition throughout the nuclear cavity, it remains so distributed for but a very short time, for nearly all of the nuclei containing the unsegmented spireme show the thread somewhat contracted to form a loose aggregation occupying the central part of the nuclear cavity (Figs. 7 and 8).

A stage possibly similar to this has been described by Davis (12) as occurring in the prophases of dividing nuclei in germinating spores of *Pellia*. Here the chromatic material becomes more or less grouped around or near the nucleole in a loose aggregation, which does not entirely fill the nuclear cavity.

Stout (44) has also called attention to such an aggregation of the chromosomes in the nuclei of root tips of *Carex*. He says,—“At one stage in the late prophases the chain of chromosomes is tightly coiled about the nucleole.”

It is my opinion that no especial significance is to be attached to this contracted condition. The more or less connected condition of the chromosomes as late as the metaphases suggests that even during the spireme stage somewhat of a reticulate condition still exists. If such were the case, the uniform distribution of the spireme would probably be much restricted.

Upon the segmentation of the spireme, the chromosomes come to be quite widely separated from one another, causing this to be a conspicuous

phase. While not common in my material, this prophase was still frequent enough to be satisfactorily studied. The chromosomes are at first elongated rod-like structures (Figs. 9, 10, and 13). They shorten and thicken until their diameter is probably twice that of the chromosome at the time of the segmentation of the spireme, while their length becomes about twice their final diameter (Figs. 11, 12, 14).

The number of the chromosomes is small. Figs. 10 and 13 of nuclei immediately before metaphase and Fig. 19 of a polar view of a metaphase stage,—all made with no reference to the chromosome number, each show thirteen chromosomes. The chromosome count in other nuclei, not figured, gave twelve and thirteen as the number, and it is quite probable that thirteen is the correct number.

The details of spindle formation could not be followed on account of the minuteness of the nuclei. At the time of the metaphases, when the spindle is most conspicuous it is distinctly bipolar (Figs. 15, 16, and 18). Areas of protoplasm which suggest kinoplasmic caps were in several cases observed (Fig. 13) and it is quite probable that they give rise to the spindle in a manner quite similar to that described for root tip mitosis. An examination of many metaphases failed to reveal anything that could be interpreted as a centrosome or centrosphere. Neither in the first division of the cell nor in the second or the third division was a centrosome to be seen. The appearance of centrosome-like bodies in antheridia of some Bryophytes at the time of the formation of the male gametes suggests that centrosomes are to be expected at the time of the formation of swimming cells. If such bodies are present in *Tetraspora* I have been unable to stain them. Davis (12) has described centrosome-like bodies in *Pellia* which are usually present only in the early prophases and disappear before the metaphases. No such evanescent structure could be identified in *Tetraspora*.

The single group of chromosomes of the metaphases splits to form the two groups of the anaphases (Fig. 19). It is very probable that each chromosome splits to form the chromosomes of the anaphases,—though on account of their minuteness this could not be determined with certainty.

The reconstruction of the daughter nuclei (Figs. 20, 22, 23, and 24) as far as can be determined is the same as in higher plants. The chromatic material is at first densely massed (Fig. 20). This mass becomes looser and a nuclear membrane is formed (Fig. 22). The separation of the chromatic material into smaller and smaller parts goes on till the reticulum is formed (Figs. 23 and 24). Although the second and third nuclear and cell-divisions of the vegetative cell to form the eight gametes followed the first in quick succession, nevertheless the daughter nuclei in each case enter a resting condition similar to that of the vegetative nuclei (Figs. 24 and 28).

The partition walls are formed during the telophases by means of a central spindle not greatly unlike that in dividing cells in the higher plants.

Fig. 20 shows a definite central spindle in a very early telophase. Later, as in Fig. 23, the rudiments of a cell-plate may be seen in the central part of the central spindle as a collection of granules. Whether these granules arise as thickenings of the spindle fibres could not be determined with certainty. Stages such as are shown in Fig. 22, in which the central spindle and the cell-plate do not extend out to the cell-wall show clearly that cell-plate formation is initiated between the daughter nuclei, and the wall extends out to the wall as is the case in the higher plants. This, as above mentioned, is the case in *Oedogonium*, and may be of common occurrence in the green Algae, though in *Spirogyra* and *Cladophora* the cross wall formation is initiated at the wall and extends inward.

The splitting of the cell-plate seems also to be from the centre outward (Figs. 25 and 26). Frequent cases were observed in which there was a widely separated cleft between the two daughter-cells which did not extend to the surface of the cell. The wide separation of the cleft is of course due to plasmolysis. If the cell-plate had been split completely to the wall one could expect that the shrinkage would manifest itself on the outside of the cells, rounding up the newly formed corners and edges. This was not the case in any of the cells observed. It is clear that the cleavage does not begin at the surface and extend inward.

The single disc-shaped pyrenoid remains unchanged through the three nuclear and cell-divisions of the vegetative cell (Figs. 18, 27, and 28), so that at first but one of the eight cells which are to form the gametes has a pyrenoid (Figs. 28 and 29). The gametes at the time that they become motile are all equipped with a pyrenoid, the origin of which was not determined. It is clearly evident, however, that here the pyrenoid does not arise by the division of a pre-existent pyrenoid, but that it is formed anew from the cytoplasm. In the vegetative multiplication of the cells of the Alga the pyrenoid divides to form the pyrenoids of the daughter-cells.

It seems also clear that the pyrenoids do not disappear upon the initiation of the steps leading to gamete formation as is the case in *Hydrodictyon* and in *Chlamydomonas* according to Klebs (26, 27), in *Cladophora* according to Strasburger (45), and in *Volvox* on the authority of Overton (40). Fig. 29 shows the persistent pyrenoid in the eight-celled stage before the differentiation of the gametes.

The pyrenoids commonly appear as flattened disc-like bodies, one flattened surface being toward the nucleus. Usually there is a plane of cleavage extending through the centre of the mass parallel with the flattened surface (Figs. 8 and 9), separating it more or less completely into two parts. This apparent doubleness may be similar to that described by Timberlake (48) for *Cladophora*, in which 'the differentiation of the pyrenoid into two parts takes place in such a way as to divide it by a plane passing through its longer axis. In many cases the pyrenoid is actually split into

halves with a fairly broad cleft between them'. Not uncommonly the pyrenoid seems to be a solid mass with no plane of cleavage. Frequently they appear as rounded bodies (Figs. 13 and 14). These may in part of course be the flattened pyrenoids as seen from the side. Many of these rounded pyrenoids have undergone peripheral cleavage to form a number of smaller irregular starch masses which are perfectly distinct from one another (Fig. 31). They stain a uniform blue colour and show no differentiated central part such as is so well known for *Spirogyra* and has been described as a saffranin staining region by Timberlake (47) for *Hydrodictyon*, and by Lutman (32) for *Closterium*. They are, apparently, more like the pyrenoids of *Cladophora*, *Oedogonium*, or *Rhizoclonium* in which, according to Timberlake, 'in some instances the entire pyrenoid is converted into starch without previous cleavage.' As mentioned above, the protoplasm in which the pyrenoids lie cannot be distinguished in texture and staining reaction from that of other parts of the cell, though a narrow unstained zone is usually present immediately surrounding the pyrenoid.

DISCUSSION.

It will be seen from the foregoing that the general details of the nuclear structure and mitosis in *Tetraspora* and in other Chlorophyceae thus far investigated, are essentially the same. As far as can be determined from the minute nuclei of *Tetraspora* the structure of the resting nuclei, and the conduct of the chromatin in spireme formation, the origin and development of the spindle and the mode of the formation of the cell-plate are processes the same as in the Angiosperms. A striking uniformity thus exists throughout the green plants in the phenomena of nuclear division.

Accounts of the presence of centrosomes or centrospheres in certain cells of the Liverworts have encouraged the expectation that such centres should be common in the Chlorophyceae. Reports of great variation in the polar organization in the nuclei of the Protozoa, on the other hand, have contributed to this belief,—based on the view that certain Protozoans and the Chlorophyceae have probably arisen from a common ancestor. Investigations upon the mitosis in the green Algae have not sustained this expectation. The presence of centrosomes or centrospheres has thus far not been satisfactorily demonstrated in cells of this group of plants. It must be admitted, however, that the structures reported for *Valonia* (17), *Derbesia* (13), and *Hydrodictyon* (48, 59) strongly suggest centrosomes, and further investigations may prove them to be such. Nevertheless, they are clearly not such permanent centres as have been described by Harper (21) for *Phyllactinia*, as well as by others for various plant and animal cells.

The presence of intranuclear spindles in *Derbesia*, *Valonia*, and possibly in *Hydrodictyon* is suggestive of a permanent centre. Still, mitosis in

Oedogonium takes place without visible centrosomes though the spindle seems clearly intranuclear.

The appearance in the antheridia of certain Liverworts [*Marchantia* (22, 58), *Fegatella* (55), *Riccia* (30) and others] of centrosome-like bodies in the last cell generations before the formation of the motile gametes suggests the possibility of centrosomes appearing at or shortly before the stage at which the blepharoplast is present. While the three nuclear divisions of the vegetative cell of *Tetraspora* lead to gamete formation and should, on the above hypothesis, be especially favourable for the detection of centrosomes, nevertheless they show no such bodies.

According to the literature on mitosis in the Protozoan cell, many low forms have the chromatin of the nucleus concentrated in the nucleole. Such phenomena have been reported for *Hydrodictyon* and *Sphaeroplea* by Golenkin (19) and have been expected by some in other genera of the lower green Algae. Golenkin's results are probably to be explained as due to improper fixation or staining, since both Timberlake and Yamanouchi have demonstrated the presence of a reticulum in *Hydrodictyon*. Further research with *Sphaeroplea* will probably give similar results. It appears then that *Spirogyra* alone among the green plants thus far investigated is still in doubt. If we attempt to explain this divergence of the mitosis in *Spirogyra* on the ground that the Conjugatae probably arose contemporaneously with the Chlorophyceae from a common ancestor, and have developed their mode of mitosis independently of the latter group, we are confronted with the facts that in *Zygnema* and *Closterium* the nuclear division is well proved to be similar to that in the higher plants. The great uniformity in the nuclear phenomena of the line of green plants beginning with the Chlorophyceae leads one to expect a similar uniformity in the Conjugatae. According to the literature on this latter group such uniformity does not exist. Contrary to Moll's suggestion, this lack of uniformity probably is to be found in the investigator and his methods of fixation and staining, &c., rather than in the different species of *Spirogyra*. This conclusion is borne out by the fact that different investigators interpret differently the nuclear phenomena of the same species. For example, Moll believes that all of the chromosomes of *Spirogyra crassa* come from the nucleole, while van Wisselingh reports that but two come from the nucleole of this same species, while ten arise from the reticulum.

Dangeard's suggestion (11) that the groups Chlamydomonadaceae and Euglenidae be separated on the basis of their type of nuclear division was based upon a comparison of a number of genera of both orders. Although objection may be made to the terms 'teleomitose' and 'haplomitose' on the ground that the latter term implies too great simplicity of mitosis, it nevertheless seems clear that two perfectly distinct types of nuclear division exist here. In the Chlamydomonadaceae there are no centrosomes and the

spindle is of the same general type which is found in the cells of the higher plants. In the Euglenidae, on the other hand, no recognizable spindle threads seem to exist, but nuclear division appears to be accomplished by means of a persistent 'nucleo-centrosome' which divides, moves to the poles of the cell—each half with a group of chromatic bodies surrounding it. It is of interest here to note that this latter type of nuclear division, although occurring in the Diatoms and possibly in the Myxomycetes, has not been reported for any of the line of green plants arising with the Chlorophyceae.

In view of the remarkable similarity in nuclear structure and division among all green plants, this wide difference in the character of the mitosis in these two orders makes it clear that no closer relationship exists between them than that due to a possible common origin from a remote ancestor. The *Euglena* type of nuclear division has not been reported for any green plant. It must be admitted, nevertheless, that Olive's figures of mitosis in *Empusa* (38) show a striking similarity to the *Euglena* type of mitosis. This close similarity in the mitosis of such unrelated forms may suggest that data of a mitotic nature has little phylogenetic significance. The type of nuclear division so characteristic of the green plants is, however, so constant that it seems impossible to find an easy transition from it to the type characteristic of the Euglenidae. Thus the origin of the Chlamydomonadaceae from the Euglenidae as suggested by Blackman and others seems excluded.

It seems clear that in *Tetraspora*, although the chlorophyll-bearing protoplasm is definite and constant with regard to the position of the nucleus and pyrenoid, it is, nevertheless, not a highly differentiated protoplasmic body such as is to be seen in the plastids of *Spirogyra*, *Oedogonium*, *Vaucheria*, and the higher green plants. The chlorophyll-bearing area does not differ in texture or staining reaction from the other protoplasm. This is not in accord with Dangeard's observations in *Chlamydomonas* (9) in which he believes that the chlorophyll-bearing protoplasm is alveolar, while that surrounding the nucleus is reticulate. Timberlake (47) believes that the protoplasm in *Hydrodictyon* is not segregated into a 'layer containing chlorophyll and one containing nuclei'. The form of the cells of this Alga prevents, however, positive proof of this point with living material, while with fixed material it is possible that, as is the case with *Tetraspora*, no distinction can be detected. It is not improbable, as Timberlake says, that more careful investigation of a large number of forms of the Chlorophyceae will reveal frequent cases in which highly differentiated chloroplastids are not present.

SUMMARY.

1. The nucleus of *Tetraspora* in the resting condition has a chromatic reticulum, net knots and nucleole distributed in the same manner as in the higher plants.
2. A definite spireme is formed from the reticulum. The spireme segments to form about thirteen chromosomes.
3. The nucleole shows no signs of disintegration until the increase in chromatic material has come to an end.
4. Centrosomes are not to be identified at any stage of the nuclear division.
5. Cell-division is accomplished by the splitting of a granular cell-plate which has been formed by the central spindle. The splitting takes place from the centre outward.
6. The entire pyrenoid segments to form several starch bodies. No differentiated central area is present.

UNIVERSITY OF TEXAS,
February 15, 1913.

LITERATURE CITED.

1. ALLEN, C. E.: Die Keimung der Zygote bei *Coleochaete*. Ber. d. deutsch. bot. Ges., xxiii, 1905, pp. 285-92.
2. BERGHS, J.: Le noyau et la cinèse chez le *Spirogyra*. La Cellule, xxiii, 1906, pp. 53-86.
3. BLACKMAN, F. F.: The Primitive Algae and the Flagellata: an Account of Modern Work bearing on the Evolution of the Algae. Annals of Botany, xiv, 1900, pp. 647-88.
4. CALKINS, G. N.: The Phylogenetic Significance of certain Protozoan Nuclei. Ann. N. Y. Acad. Sci., xi, 1898, pp. 379-400.
5. —————: Mitosis in *Noctiluca*. Ginn & Co., Boston. Also Journal of Morph., xv, 1898, pp. 711-72.
6. CHODAT, R.: Algues vertes de la Suisse: Pleurococcoïdes—Chroolepoïdes. Beitr. z. Kryptogamenflora der Schweiz, Band i, Heft 3, 1902.
7. —————: Étude critique et expérimentale sur le polymorphisme des Algues. Mém. publ. à l'occasion du Jubilé de l'Université de Genève. 1909.
8. COLLINS, F. S.: The Green Algae of North America. Tufts College Studies, vol. ii, No. iii, Scientific Series.
9. DANGEARD, P. A.: Mémoire sur les Chlamydomonadinées, ou histoire d'une cellule et théorie de la sexualité. Le Botaniste, vi, 1898, pp. 65-292.
10. —————: Étude sur la structure de la cellule et ses fonctions. Le *Polytoma uvella*. Le Botaniste, viii, 1901, pp. 1-58.
11. —————: Recherches sur les Eugléniens. Le Botaniste, ix, 1902, pp. 1-261.
12. DAVIS, B. M.: Nuclear studies on *Pellia*. Annals of Botany, xv, 1901, pp. 147-80.
13. —————: Spore-formation in *Derbesia*. Annals of Botany, xxii, 1908, pp. 1-20.
14. DEBSKI, B.: Beobachtungen über Kerntheilung bei *Chara fragilis*. Jahrb. wiss. Bot., xxx, 1897, pp. 227-48.

15. DEGAGNY, CH.: Sur les matières formées par le nucléole chez le *Spirogyra setiformis*, &c. Compt. Rend., 1893, pp. 269-72.
16. ———: Sur la concordance des phénomènes de la division du noyau cell. chez les Lis et chez les Spirogyras, etc. Compt. Rend., 1893, pp. 1397-1400.
17. FAIRCHILD, D. G.: Ein Beitrag zur Kenntniss der Kerntheilung bei *Valonia utricularis*. Ber. d. deutsch. bot. Ges., xii, 1894, pp. 331-8.
18. GAY, F.: Recherches sur le développement et la classification de quelques algues vertes. Paris, 1891, 119 pp.
19. GOLENKIN, M.: Ueber die Befruchtung bei *Sphaeroplea annulina*. Bull. Soc. imp. Moscou, 1899, pp. 343-61.
20. GRUBER, A.: Beiträge zur Kenntniss der Physiologie und Biologie der Protozoen. Ber. d. Naturf. Ges. zu Freiburg, i, 1886, pp. 33-56.
21. HARPER, R. A.: Sexual Reproduction and the Organization of the Nucleus in certain Mildews. Publ. Carnegie Inst., Washington, No. 37, 1905.
22. IKENO, S.: Die Spermatogenese von *Marchantia polymorpha*. Beih. z. Bot. Centralblatt, xv, 1903, pp. 65-88.
23. ISHIKAWA, C.: Studies on Reproductive Elements: II. *Noctiluca miliaris*, Sur., its division and spore formation. Journ. College of Sc. Imp. Univ. Japan, vi, 1894, pp. 297-337.
24. ———: Further Observations on the Nuclear Division of *Noctiluca*. Journ. Coll. Sci. Tokio, xii, 1899, pp. 243-62.
25. KARSTEN, G.: Ueber die Reduktionstheilung bei der Auxosporenbildung von *Surirella saxonica*. Zeitschr. f. Bot., iv, 1912, pp. 417-26.
26. KLEBS, G.: Ueber die Bildung der Fortpflanzungszellen bei *Hydrodictyon*. Bot. Zeitung, xlix, 1891, p. 789.
27. ———: Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena, 1896.
28. KEUTEN, J.: Die Kerntheilung von *Euglena viridis*. Zeitschr. f. wiss. Zool., lx, 1895, pp. 215-35.
29. LAUTERBORN, R.: Untersuchungen über Bau, Kerntheilung und Bewegung der Diatomeen. Leipzig, 1896.
30. LEWIS, C. E.: The Embryogeny and Development of *Riccia lutescens* and *R. crystallina*. Bot. Gaz., xli, 1906, pp. 109-38.
31. LUTMAN, B. F.: Cell and Nuclear Division in *Closterium*. Bot. Gaz., li, 1911, pp. 401-30.
32. ———: Cell Structure of *Closterium Ehrenbergii* and *Closterium moniliferum*. Bot. Gaz., xlix, 1910, pp. 241-55.
33. MEUNIER, A.: Le nucléole de *Spirogyra*. La Cellule, iii, 1887, pp. 333-410.
34. MITZKEWITSCH, L.: Ueber die Kerntheilung bei *Spirogyra*. Flora, lxxxv, 1898, pp. 81-124.
35. ———: Zu der Frage nach der Zell- und Kerntheilung von *Oedogonium*. Ref. Strasburger, Hist. Beitr., vi, 1900, p. 191. (In Russian.)
36. MOLL, J. W.: Die Fortschritte der mikroskopischen Technik seit 1870. Prog. Rei Bot., ii, 1908, pp. 227-91.
37. NÉMEC, B.: Ueber die Kerntheilung bei *Cladophora*. Bull. Acad. Internat. d. Sci. de l'Emp. François Joseph, Classe Scient., Math., etc., xv, 1910, pp. 50-55.
38. OLIVE, E. W.: Cytological Studies on the Entomophthoreae. II. Nuclear and Cell-division of *Empusa*. Bot. Gaz., xl, 1906, pp. 229-61.
39. OLTMANN, F.: Morphologie und Biologie der Algen. Jena, 1904.
40. OVERTON, E.: Beitrag zur Kenntniss der Gattung *Volvox*. Bot. Centralbl., xxxix, 1889, pp. 145-50.
41. REINKE, J.: Ueber *Monostroma bullosum*, Thur. und *Tetraspora lubrica*, Ktz. Jahrb. wiss. Bot., xi, 1878, pp. 531-47.
42. SCHAUDINN, F.: Ueber das Centalkorn der Heliozoen. Verh. d. zool. Ges., vi, 1896, pp. 113-30.
43. SCHEWIAKOFF, W.: Ueber die karyokinetische Kerntheilung bei *Euglypha alveolata*. Morph. Jahrb., xiii, 1888, pp. 193-258.
44. STOUT, A. B.: The Organization of the Chromosomes in *Carex*. Science, N.S., xxxiii, 1911, pp. 264.
45. STRASBURGER, E.: Zellbildung und Zelltheilung. 3. Aufl., Jena, 1880.
46. ———: Ueber Kern- und Zelltheilung im Pflanzenreich. Jena, 1888.

47. TIMBERLAKE, H. G.: Starch formation in *Hydrodictyon utriculatum*. Ann. Bot., xv, 1901, pp. 619-35.
48. ———: Development and Structure of the Swarmspores of *Hydrodictyon*. Trans. Wis. Acad. Sci. Arts and Letters, xiii, 1902, pp. 486-522.
49. ———: The Nature and Function of the Pyrenoid. Science, N.S., xvii, 1903, p. 460.
50. TUTTLE, A. H.: Mitosis in *Oedogonium*. Journ. Exp. Zool., ix, 1910, pp. 143-57.
51. WEST, G. S.: A Treatise on the British Fresh-water Algae. Cambridge, 1904.
52. WILLE, N.: Algolog. Mittheilungen, LV; Ueber die Zelltheilung bei *Oedogonium*. Jahrb. wiss. Bot., xviii, 1887, pp. 426-518.
53. ———: Nachträge zum I. Teil, Abteilung 2. (Die natürlichen Pflanzenfamilien, Engler und Prantl.) Conjugatae und Chlorophyceae. Leipzig, 1909.
54. WILSON, E. B.: The Cell in Development and Inheritance. New York, 1906.
55. WILSON, M.: Spermatogenesis in the Bryophyta. Ann. Bot., xxv, 1911, pp. 416-57.
56. VAN WISSELINGH, C.: Ueber Kerntheilung bei *Spirogyra*. Flora, lxxxvii, 1900, pp. 353-77.
57. ———: On the Structure of the Nucleus and Karyokinesis in *Closterium Ehrenbergii*, Men. Koninkl. Akad. Wetens. Amsterdam, xiii, 1910, pp. 365-75.
58. WOODBURN, W. L.: Spermatogenesis in certain Hepaticae. Ann. Bot., xxv, 1911, pp. 299-313.
59. YAMANOUCHI, S.: *Hydrodictyon africanum*, a new species. Bot. Gaz., lv, 1913, 74-9.
60. ZACHARIAS, E.: Ueber den Nucleolus. Bot. Zeit., xliii, 1885, pp. 257, 273, 289.

EXPLANATION OF FIGURES IN PLATE LVI.

Illustrating Mr. McAllister's paper on *Tetraspora lubrica*.

All figures were drawn with the aid of a camera lucida, a Zeiss apochromatic immersion lens, 1.40 N.A., and an 18 compensating ocular (magnification about 3,000) being used in all cases except for Fig. 1, which has a magnification of about 1,650 diameters.

- Fig. 1. Cells drawn from living material showing localized chlorophyll-bearing area.
- Fig. 2. Cells showing nucleus in resting condition. The cytoplasm here shows no differentiation into chloroplastid.
- Fig. 3. A resting nucleus showing reticulum and nucleole.
- Fig. 4. An early prophase showing increase of chromatic material in the nucleus.
- Fig. 5. A later prophase.
- Fig. 6. Still later prophase with nucleole still intact.
- Fig. 7. The chromatic material has contracted to form a knot in the centre of the nuclear cavity.
- Fig. 8. A looser knot showing the presence of a spireme. The double nature of the pyrenoid is well shown in this figure.
- Fig. 9. The segmented spireme.
- Fig. 10. As above.
- Fig. 11. The chromosomes shortening and thickening.
- Fig. 12. Similar to Fig. 11.
- Fig. 13. Immediately before metaphase of the second division. Caps of denser protoplasm are to be seen at the poles of the nucleus. Thirteen chromosomes are to be identified.
- Fig. 14. Late prophase of the second division.
- Fig. 15. Metaphase of the first division.
- Fig. 16. Metaphase of the first division.
- Fig. 17. Metaphase of the second division. One a polar view with eleven chromosomes.
- Fig. 18. A polar view of the first metaphase showing thirteen chromosomes.
- Fig. 19. Anaphase of the first division.
- Fig. 20. Late anaphase or early telophase—the central spindle conspicuous.
- Fig. 21. Early telophase of the second division.

Fig. 22. Later telophase—the nuclear membrane is now present and the cell-plate partly formed.

Fig. 23. The cell-plate appears as a definite line of granules, more conspicuous in the central region, but to be traced indistinctly nearly to the cell-wall. Part of the central spindle is still easily identified.

Fig. 24. Fully formed daughter nuclei have moved closer together. The kinoplasm of the central spindle area no longer appears fibrous. The cell-plate is clearly most perfectly developed in the central region.

Figs. 25, 26. The cell-plate is split in the middle and widely separated by plasmolysis. The cleavage cannot be traced to the peripheral wall.

Fig. 27. Completely separated daughter-cell of the first division.

Fig. 28. Daughter nuclei of the third division. One single large pyrenoid.

Fig. 29. The eight cells at the close of the third division. A single deeply lying pyrenoid is to be seen in the central cell.

Fig. 30. A gamete rounded up—the eye spot and cilia not yet visible.

Fig. 31. Pyrenoids showing peripheral cleavage to form several starch masses.

16.

17.

18.

19.

20.

21.

22.

23.

24.

25.

26.

27.

28.

29.

30.

Hath lith et imp.

McAllister, F. 1913. "Nuclear division in *Tetraspora lubrica*." *Annals of botany* 27, 681–696. <https://doi.org/10.1093/oxfordjournals.aob.a089483>.

View This Item Online: <https://www.biodiversitylibrary.org/item/237410>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a089483>

Permalink: <https://www.biodiversitylibrary.org/partpdf/319996>

Holding Institution

Smithsonian Libraries

Sponsored by

Biodiversity Heritage Library

Copyright & Reuse

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

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