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A critical study of the chromosomes in two species of myxophyceae

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A CRITICAL STUDY OF THE CHROMOSOMES IN TWO SPECIES OF MYXOPHYCEAE

by

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INTRODUCTION

The Myxophyceae or blue-green algae have been frequent subjects for cytological investigation since Schmitz (1879) first called attention to two regions in the protoplast. Since that publication the morphological and chemical nature of the genetic material and the mechanism of cell division in the Myxophyceae has been a controversial subject. The evidence that has been presented is, for the most part, more voluminous than enlightening.

In view of the great diversity of opinion and interpretation it is difficult to avoid the impression that faulty or nonspecific cytological methods have been responsible, in large measure, for the confusion that exists. It is clear that the material is difficult to handle because of its small size and complex structure.

A striking feature of many of the papers on the cytology of the Myxophyceae has been the fine literature reviews. Adequate reviews for the various periods are found in Phillips (1903) 1879-1901, Spearing (1937) 1901-1933, and Fritsch (1945) 1879-1940. The following is the more important recent literature since 1940. Hollande (1946) in a study of Phormidium uncinatum, P. retzii, P. tenue and Nostoc verrucosum proposed the term 'prospiremoide' for the central body. The 'prospiremoide' is homologous to the nucleus of higher

organisms. It differs from the 'spiremoide' (spiremoide Fr. = spireme, Hollande is apparently refering to the obsolete spireme concept of the nucleus as used by classical authors.) of other nuclei by the presence of volutin granules. The 'spiremoide' is formed by individual tubules which surround a nucleoplasm. Finally he concluded that the cytological structure of the Cyanophyceae is fundamentally similar to that of other plants and to animals. Strickland (1948) first applied a smear technique to certain Myxophyceae and reported the chromatin material in Myxophyceae to be chemically and morphologically similar to the chromosomes of higher plants. Drawert (1949) using the Feulgen nuclear reaction in Oscillatoria Borneti found a reticular 'chromidialapparat' which stained positively and in addition some small granules in the cytoplasm which also reacted. He concluded that a morphologically differentiated nucleus is not present in the Cyanophyceae examined but rather a Feulgen positive 'chromidialapparat' is present in a granular or reticular form and lying mostly in the middle of the cell embedded in the plasma ground substance. Bringmann (1950) denoted the chromatic material as a 'karyoide' which he distinguished from the nucleus of higher organisms by the manner of occurrence and synthesis of the nucleic acid systems and cell division. He concluded that the chromatic substance does not possess the organization

of a nucleus but, however, functions as one. Bringmann (1953) observed that following treatment with lanthanum acetate positive staining reactions for deoxyribonucleic acid (DNA), ribonucleic acid (RNA), phosphate and lipids occurred. He concluded that the existence of this group of substances makes it possible to relate the cell center of the Cyanophyceae to mitochondria of higher organized cells. Zastrow (1953) utilized basic vital stains in determining the basiophilic nature of a 'zentralsubstanz'. The presence of the two nucleic acids characterizes the 'zentralsubstanz' as a nuclear equivalent, appearing in cells capable of dividing, in the form of a continuous body (central body). With increasing cell age individual particles can be recognized in the 'zentralsubstanz'. A centroplasm could not be found. Biswas (1953) noted that the central body of the Myxophyceae has a greater affinity for pyronin stain than methyl green. He stated that this indicated the presence of RNA or DNA in a less polymerized form. Cassel and Hutchinson (1954) working with several smaller Myxophyceae concluded that the Myxophyceae possess material which is clearly of a nuclear nature, although they presented no new evidence in support of this conclusion. Drews and Niklowitz (1956) working with Phormidium uncinatum observed that the centroplasm contains, in diffuse distribution, RNA and three different granular-like elements: (1)

small, mostly netlike, Feulgen positive granules; (2) large, scattered granules, probably of condensed phosphate and eventually containing also RNA; (3) small, peripheral situated granules, containing phospholipids. Other granules, with mitochondrial function, could be detected near the transverse walls. This is perhaps the first report of mitochondria, in the form of granules, occurring in the Myxophyceae. Fritsch (1945) reported that mitochondria were lacking in the Myxophyceae. Bringmann (1953) compared the entire central body to mitochondria. Thus it would appear that a clear understanding of mitochondria in the Myxophyceae is not yet established. Biswas (1956) isolated the nucleic acids from Nostoc muscorum using Heinrich's modification of the method of Schmidt and Thannhauser. He concluded that DNA in Cyanophyceae was similar in kind to that from other sources and that the RNA of Cyanophyceae showed no qualitative deviation from that found in other forms. Further chemical studies have revealed (Biswas, personal communication) that the DNA can be divided into two fractions, one DNA-I is soluble in alkali and the other DNA-II in acids. These fractions differ in their molar constitutions of bases and also in their prior metabolic pattern. Biswas (1957) concluded on the basis of cytochemical studies on the central body that the chromatic threads in the central body show characteristics paralleled

with those of chromosomes in so far as the protein-moiety is concerned.

The present state of our knowledge allows us to consider at least three different alternatives in regard to the cell structure of Myxophyceae. The first would be to follow Fritsch (1945) who felt that the cell structure of Myxophyceae cannot be directly compared with that of other plants (except certain bacteria) and since the central body differs in many respects from a true nucleus it should not be compared with one because there is no evidence that the central body has any direct connection with a true nucleus. The second would be to follow many of the recent workers i.e. Drawert, Bringmann, and Cassel and Hutchinson who feel that the central body of the Myxophyceae is equivalent to a true nucleus although it lacks chromosomes and the precise organization. The third would be to follow the minority and call the central body of the Myxophyceae a nucleus, on the basis of certain chemical and morphological characteristics in common with those of higher organisms.

To follow the concept as exemplified by Fritsch would involve ignoring the fact that, from the chemical standpoint, the central body of the blue-green algae is similar to other nuclei.

To accept the view point that the central body of the

Myxophyceae is only equivalent to the nucleus of higher forms always seems to involve the coining of some new term to designate this structure. In view of the many terms which have been introduced and the lack of general acceptance of any of them it appears that this approach would continue to fail to contribute to the need for a clear understanding of the true nature of the central body.

Evidence, obtained from the species of Myxophyceae used in this investigation, that tends to justify the acceptance of the viewpoint that the central body is a nucleus will be given in this paper.

Possibly the best starting point for this problem is to attempt to answer the question as to whether the Myxophyceae contain true chromosomes, since the chromosomes are the most fundamental structures of all nuclei. "The nucleus is the chromosomes" (Darlington and La Cour, 1947).

Therefore the first phase of the problem is - does the genetic material in the Myxophyceae exist in the form of chromosomes? Secondly, what is the mechanics of the nuclear division in the Myxophyceae?

This investigation was undertaken in order to verify the methods and check the results obtained with the smear technique by Strickland (1948). Additional methods were also applied which might enable a more critical evaluation of the

morphology of the chromatin elements.

MATERIALS AND METHODS

The organisms used were *Calothrix parientina* Born. and Flah. and *Scytonema Hofmannii* Born. and Flah. They are representatives of the families Rivulariaceae and Scytonemataceae respectively.

The blue-green algae used were grown as unialgal pure cultures on nutrient solutions. The cultures were freed of bacteria by the method reported by Gerloff *et al.* (1950). This technique depends on the fact that the blue-green algae are slightly more resistant to ultra-violet irradiation than are bacteria. The utilization of pure material under controlled conditions of culture eliminates one of the criticisms directed at earlier workers by Fritsch (1945).

The nutrient solution used was a modified Beyerinck's prepared by diluting a standard Beyerinck's (Bold, 1942) by one-half and making it up as a ten per cent soil decoction. A small amount of calcium carbonate was added to each one hundred milliliters. The soil decoction was prepared by adding ten grams of rich soil to one hundred milliliters of water and autoclaving at fifteen pounds pressure for fifteen minutes. The supernatent liquid was then removed and filtered twice.

The modified Chu number 10 nutrient solution used by Gerloff *et al.* (1950) for the culture of blue-green algae was also used but no advantage was noted in growth over the

modified Beyerinck's solution. It should be said, however, that a critical comparison of the two nutrient solutions was not made as that was outside the scope of this problem.

The algae were grown in one hundred and twenty five milliliter Erlenmeyer flasks fitted with gauze wrapped cotton stoppers capped with aluminum foil. Approximately twenty five milliliters of nutrient solution was used in each flask. All glassware and nutrient solutions were autoclaved and aseptic conditions prevailed. Transfers were made in a transfer chamber equipped with a fifteen watt germicidial lamp. The cultures were grown at room temperature under constant illumination from a twenty watt fluorescent light suspended one foot above the flasks.

The method employed was a smear technique which has been very productive in the study of chromosomes of other organisms, and was first employed in the blue-green algae by Strickland (1948). The major difficulty in studying the genetic material in the Myxophyceae is to overcome the masking effect of the chromatoplasm on the chromatin material. It surrounds the central body, is very dense and quite commonly filled with refractive granules of various reserve materials and other metabolites. This gel-like nature of the chromatoplasm along with the minute size of the chromatin elements makes the study of an intact blue-green alga cell a difficult

task. However, by employing the smear technique the masking effect of the surrounding protoplasm is overcome and the chromatin elements can be readily seen.

The modified Feulgen reaction for small and diffuse chromatin material as given by Rafalko (1946) was used.

Heidenhain's iron alum haematoxylin was used in the following schedule:

A. Fixation with Carnoy's 3:1 acetic acid and absolute alcohol, or substitute propionic acid for the acetic acid.
B. Hydrate to water.
C. Mordant in 4% iron alum for thirty minutes.
D. Stain in 0.5% haematoxylin from thirty minutes to twenty four hours.
E. Rinse in water.
F. Differentiate with 2% iron alum or a saturated aqueous solution of picric acid.
G. Wash in running water for thirty to sixty minutes.
H. Dehydrate in alcohol series and xylol.
I. Mount in balsam.
Note: Material smears best in the 10% alcohol stage of the dehydration process.

With the haematoxylin stain the best results were obtained when the material was fixed and stained in bulk in small glass vials. The smearing was done after dehydration to around ten per cent alcohol.

The acid Giemsa method as given by Wolcott (1954) was used with the following slight modification: fixation was with Carnoy's fluid instead of with osmium tetroxide and the Duboscq-Brasil fluid was omitted. The chromatin material is stained red to red-purple and the cytoplasm is colorless or

with a slight tinge of blue.

To unravel the chromatin elements, films of material on clean slides were exposed to NH_4OH fumes for 15-30 seconds before fixation after the method as given by Kuwada and Nakamura (1934).

The experiment by Mazia and Dan (1952) in which they isolated the mitotic apparatus of sea urchin eggs by the use of detergents which solubilized other cell components was thought to be a possible avenue for isolating the chromatic material of the blue-green algae. Although it was realized that many additional problems of fixation and penetration might be encountered with plant material covered by sheath and cell walls incontrast to the relatively thin membranes of sea urchin eggs.

The schedule of treatment with detergents:

A. Fixation with 30% ethanol at - 10 degrees Centigrade.
B. Add equal volume 12-15% peroxide for 30 minutes at room temperature.
C. Detergent: add 20-25 volumes 1-2% detergent in water for thirty minutes up to seven days with constant stirring. The two detergents used were Duponol D and digitonin.
D. To collect mitotic apparatus centrifuge at 500 g. for three minutes.
E. Re-suspend in water and store at refrigerator temperatures (10-15 degrees Centigrade).
F. An alternate method was a low concentration of peroxide where the 15% peroxide is replaced by 3-4% peroxide.
G. In long detergent treatment the detergent was changed each hour and accompanied by

vigorous shaking.

All observation and photographs were made with a Spencer 2mm Numerical Aperature of 1.40 apochromat oil immersion lens. The substage condenser was fitted with a lens of Numerical Aperature 1.40. The light source was a Spencer AO-735-D 100 watt filament lamp. The film used for all photographs was Kodak Contrast Process Ortho. The film was developed with Kodak D-II, and the prints were developed with Kodak dektol. The film and prints were fixed with Kodak Acid Fixer.

RESULTS

The application of the method of Mazia and Dan (1952) failed to disperse the cytoplasm of the cells of C. parientina Born. and Flah. and S. Hofmannii Born. and Flah. It was also applied to the cells of onion root tips for comparison but no isolation of the mitotic apparatus occurred. The time of treatment with detergent was increased by intervals up to seven days. After prolonged treatment with detergents the material was partially macerated but no isolation of any structure resembling a mitotic apparatus or chromatin elements occurred. Since any mitotic apparatus that might be present would be extremely small it was thought that staining the material would make it easier to observe. Therefore Feulgen's reaction and Heidenhain's haematoxylin procedures were carried out on the material that had been treated with detergents. The Feulgen reaction proved unsuccessful on all detergent treated material. The haematoxylin stain gave good results but no separation of a mitotic apparatus was observed.

A positive staining reaction was obtained with the Feulgen method used, after conventional fixation, in the blue-green algae examined. The results, however, indicate that this reaction can be applied only as a means for the chemical identification of DNA as it is not deep enough to be useful as a stain in the study of the morphology of the chromatin

elements.

The chromatin elements of the Myxophyceae examined were observed to react to the ammonia fumes in a manner similar to the chromosomes of other organisms.

The results of the smear technique are given in the photomicrographs in Plates I - IV. The chromatin elements are seen to appear in the form of cylindrical bodies of solid consistency that stain intensely with haematoxylin and acid Giemsa. The smear technique disrupts the cells and the chromatin elements are displaced outside of the cell. The chromatin elements appear as longitudinal rods that exhibit the characteristic spiraling or coiling pattern of chromosomes. The effect of ammonia fumes in relaxing the coiled chromonemata is seen when comparison is made between figure 2, which shows chromosomes which have not been treated with ammonia fumes, and figure 3 in which the chromosomes were treated with ammonia fumes before fixation. The untreated chromosomes appear as tightly coiled rods whereas the treated chromosomes appear as loosely coiled rods. The same thing is shown with another species in a comparison of figures 4 and 5.

DISCUSSION

The existence of a nucleus in the blue-green algae has long been denied, mainly upon the evidence from a number of investigators, that the constituent parts of a nucleus cannot readily be demonstrated in preparations fixed and stained according to standard cytological procedures. It has been established that the protoplasm of the blue-green algae is differentiated into a peripheral cytoplasm and a central region in which is found one or more relatively large structures that differ from the cytoplasm in texture and in giving a positive Feulgen reaction. The existence of these entities, whose reactions toward various nuclear stains has earned them such names as 'karyoids' or 'chromatin bodies', is not in doubt, but uncertainty persists concerning their relationship to the nuclei and chromosomes of higher organisms. The condition most frequently observed has been a reticular structure with embedded granules that stain positively in the Feulgen reaction. The present study is concerned with the morphology of these granules.

A nucleus has been looked for in the blue-green algae since Schmitz recognized what he believed to be one 78 years ago. A year after this observation, however, Schmitz as a result of further work changed his opinion about the presence of a nucleus in the Myxophyceae.

Although much work has been done and an extensive literature has accumulated on the cytology of the central body investigators are still far from agreeing about its structure and behavior. The causes of this situation are many and interpretations, rather than facts, have remained controversial. Perhaps the basis for most of this disagreement over interpretations reflects the absence of a common terminology. Therefore the chromatin structures of blue-green algae will be discussed from the point of view of general cytology so that this handicap can be overcome.

Belar (1926) defined the nucleus as "any formation surrounded by cytoplasm from which chromosomes arise during division". Chromosomes in this terminology are the essential and irreducible elements of nuclei, being complex chemically and morphologically and more easily recognized by their characteristic behavior in nuclear division. Among the most important features of chromosomes are their individuality, differentiation along the long axis, occurrence in sets of nearly constant numbers, a cycle of spiralization and relaxation during nuclear division and finally their capacity of self-duplication. This is the morphological concept of the chromosome and it can be readily confirmed by observation on stained material. Throughout living organisms the chromosomes show a uniformity in chemistry and mechanics which is now

known to be the foundation of their physiological and genetical uniformity (Darlington and La Cour, 1947).

This morphological definition must now take into account the fact that chromosomes contain compounds of DNA. This feature is so common that the presence of DNA must now be regarded as a prominent aspect of chromosomes and nuclei.

The chemistry of the genetic material in the Myxophyceae has become increasingly clearer since Poljansky and Petruschewsky (1929) first obtained a positive Feulgen reaction indicating the presence of DNA. Since that time this reaction has been duplicated on a variety of blue-green algae by many different investigators. In a paper that seems to have attracted little attention, Mockeridge (1927) extracted all the constituent radicles of the nucleic acids although he could not isolate them as such from Nostoc sp. Biswas (1956) used standard methods to isolate the nucleic acids from Nostoc muscorum, and he concluded that DNA in Cyanophyceae was similar in kind to that from other organisms and that the RNA of Cyanophyceae showed no qualitative deviation from the normal type. Biswas (1957) as the result of cytochemical studies concluded that "-- the chromatin threads in the central body show characteristics paralleled with those of chromosomes, so far as the protein-moiety is concerned".

Thus the chemical nature of the genetic material in the

Myxophyceae is similar to that of higher organisms.

The question now arises as to whether this chemical similarity is correlated with a morphological structure that can be strictly compared with chromosomes of other organisms.

Scott (1887) was perhaps the first to report what he believed to be chromosomes in the Myxophyceae. There have been others who believed that they recognized chromosomes in the blue-green algae and Fritsch (1945) listed most of these. However, as noted by Fritsch and others those who have believed that they recognized chromosomes in the blue-green algae have furnished unsatisfactory supporting evidence for this observation. Most of the recent workers have given the chromatin material in the Myxophyceae some new name which is supposed to characterize it, more or less, as a nuclear equivalent. An exception to this has been Strickland (1948) who obtained photomicrographs of the chromatic elements of the blue-green algae which showed the coiling pattern characteristic of chromosomes. As a result of his observations he concluded that the chromatin in Myxophyceae is chemically and morphologically similar to the chromosomes of the higher plants. The results of the present investigation have verified the presence of the coiling pattern of the chromatin elements of the blue-green algae. This coiling pattern is affected by NH_4OH fumes in the same manner as the chromosomes

in higher organisms, since after exposure to NH_4OH fumes the tight coils of the chromatin elements relax into a very loose coiling pattern. Thus the position is taken that the chromatin of Myxophyceae is in the form of chromosomes which do not differ, chemically or morphologically, from chromosomes of higher plants.

Fogg (1956) stated that "-- it is clear that they (Myxophyceae) have neither nuclear membranes nor bodies strictly comparable with the chromosomes of other organisms". In view of the evidence presented it would seem that the blue-green algae do have bodies strictly comparable with chromosomes of other organisms. Stern (1956) doubts that the blue-green algae have evolved to a common level of chromosome organization. This doubt is no longer tenable. Robinow (1956), in an excellent review on the chromatin bodies of bacteria, stated that the blue-green algae lack visible chromosomes and share with the bacteria the "distinction" of having nonchromosomal chromatin organs. Further, although he clearly points out the need for a common terminology in the bacteria, he seems satisfied to apply to the central body of the blue-green algae the "distinctive name chromatic bodies". The author feels that the evidence presented here is sufficient to cause rejection of the idea that the blue-green algae are nonchromosomal.

The series of modifications which the nucleus ordinarily undergoes in dividing is accomplished in several different ways. The common method of division of higher plants and animals is that of mitosis, which Flemming described in 1882. The significance of this process is the duplication and orderly segregation of the chromosome set of a given nucleus. In the usual form of mitotic division the chromosomes undergo a linear contraction with a corresponding increase in thickness and stainability, arrange themselves on a fibrous spindle and undergo duplication. The longitudinal halves separate, are directed toward their respective poles where daughter nuclei form. Several variations may occur in this form of nuclear division. The divided chromosomes may fail to form spirals. The two features common to all the variations are: the production of separate recognizable chromosomes either with or without a spindle and a nondividing nucleus with its chromosomes visibly, or more frequently invisibly, clustering together in a random arrangement.

The central body of the blue-green algae has been reported to undergo division by gradual constriction and some investigators have reported a true mitosis. However, interpretations of the mode of division remain controversial. The author has observed the chromosomes of the blue-green algae studied here to undergo a division process but the true nature

and sequence of events in this cycle was not clear. It is hoped that continued work may produce a better understanding of the mechanics of this nuclear division, since none of the above types seem to apply here.

The appearance of the interphasic or resting nucleus as it appears in other organisms has only rarely been observed in the blue-green algae selected for this study. However, there have been cases where the central body appeared homogenous and surrounded by a definite membrane. These appearances were seen in intact-cell mounts with acid Giemsa.

At the present time one can only speculate as to whether the blue-green algal central body, because of chemical and structural similarities, exactly corresponds to the nucleus of higher organisms. The presence of chromosomes can no longer be doubted but there remains the necessity of the elucidation of the chromosome cycle to remove all doubt that the Myxophyceae contain a nucleus of comparable structure and function to that found in higher plants.

SUMMARY

The organisms investigated were: Calothrix parientina Born. and Flah. and Scytonema Hofmannii Born. and Flah. They are representatives of the families Rivulariaceae and Scytonemataceae respectively.

The chromatin material in these forms is Feulgen positive. It stains readily with Heidenhain's haematoxylin and acid Giemsa.

The chromatin is in cylindrical, rod-shaped bodies which show the spiral or coiled pattern characteristic of chromosomes.

The effect of ammonia fumes on these coils is the same as on the coils of chromosomes of higher plants.

It is clear from the results presented that the chromatin in the species of Myxophyceae investigated is in a form which does not differ from the chromosomes of higher plants in physical or chemical structure.

The evidence from this investigation tends to add weight to the belief that the central body of the Myxophyceae can be considered a nucleus with definite chromosomes.

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PLATE I

Calothrix parientina Born. and Flah.

Fig. 1. Smear showing typical chromosomes. Cells stained with Heidenhain's iron alum haematoxylin. X1800.

PLATE I

Fig. 1

PLATE II

C. parientina Born. and Flah.

Fig. 2. Smear showing chromosome with three tight coils. Stained with Heidenhain's iron alum haematoxylin. x1800.

Fig. 3. Smear showing chromosome with three loose coils. Treated with ammonia fumes and stained with Heidenhain's iron alum haematoxylin. x1800.

PLATE II

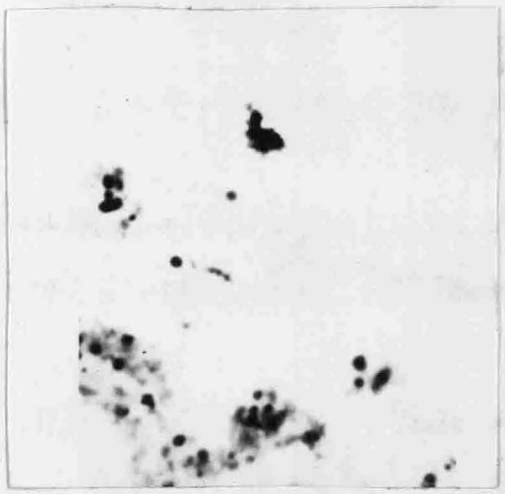

Fig. 2

Fig. 3

PLATE III

Scytonema Hofmannii Born. and Flah.

Fig. 4. Smear showing chromosome with three tight coils. Treated with ammonia fumes and stained with Heidenhain's iron alum haematoxylin. x1800.

Fig. 5. Smear showing chromosomes with loose coils. Treated with ammonia fumes and stained with Heidenhain's iron alum haematoxylin. x900.

PLATE III

Fig. 4

Fig. 5

PLATE IV

C. parientina Born. and Flah.

Fig. 6. Intact cell showing two chromosomes crossed. Stained with acid Giemsa. x1800.

Fig. 7. Intact cell showing chromosomes almost filling entire length of cell. Treated with ammonia fumes and stained with acid Giemsa. x1800.

Fig. 6

Fig. 7

VITA

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