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MITOSIS IN FUCUS

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(WITH PLATES VIII–XI)

INTRODUCTION

The first cytological study of Fucus is that of FARMER and WILLIAMS (**8**), published about ten years ago as a preliminary note. The next year STRASBURGER'S paper (**24**) on *Kerntheilung und Befruchtung bei Fucus* appeared. In 1898 the final paper of FARMER and WILLIAMS (**9**) was published.

FARMER and WILLIAMS' material, *Fucus platycarpus*, *F. serratus*, and *F. vesiculosus*, was collected on the coast of England. Their account deals with egg-formation in the oogonium, particular attention being devoted to the third division; also with fertilization and early segmentation divisions. *Ascophyllum nodosum* and *Pelvetia canaliculata* were used in a supplementary way. STRASBURGER'S work was based chiefly on material from Heligoland, Germany; in it he describes in detail the third division in the oogonium of *Fucus platycarpus* and the fertilization processes in *F. serratus* and *F. vesiculosus*.

To the brilliant results of these authors we owe most of our present knowledge of the cytology of these forms. Since the work of FARMER, WILLIAMS, and STRASBURGER, cytological conditions have been studied in a few algae, such as Dictyota (WILLIAMS **27**), Nemalion (WOLFE **28**), and Polysiphonia (YAMANOUCHI **29**). The morphology of chromosomes, in connection with the theoretical problem of alternation of generations, especially in the algae, is becoming a more important problem. For the solution of such a problem, there must be a

thorough study of the life-cycle of chromosomes; unfortunately not much of such work has been done in algae. The work of FARMER, WILLIAMS, and STRASBURGER dealt only slightly with the first two mitoses in the oogonium, where it was inferred, but not actually observed, that the reduction of chromosomes takes place. Mitoses in the antheridium, from the first division to the development of mature sperms, were not studied. In the present investigation, special attention is paid to the behavior of chromosomes in the first and second mitoses in the oogonium, and to mitoses in the antheridium.

The results here presented are based upon a study of *Fucus vesiculosus* L. Material was collected and fixed at Woods Hole, Mass., during the latter part of March and early April, 1908. As fixing reagents, Flemming's weak solution containing osmic acid, with various modifications, proved to be most satisfactory. There are several points of interest and importance in regard to the relation between the frequency of mitotic figures and environmental conditions, both in the oogonium and antheridium and in the young thallus. In general, the plants collected one or two hours after being covered by the tide were full of figures. Material was imbedded in paraffin with a melting point a little less than 52° C. Sections were cut 3 μ, sometimes 5 μ thick. Flemming's saffranin and Heidenhain's iron alum hematoxylin, with or without counterstains, were mostly used in the slides from which the accompanying illustrations were made.

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MITOSES IN VEGETATIVE CELLS OF THE THALLUS

Any young part of the thallus, when well fixed, showed figures available for study, especially in the apical region, in the adventitious outgrowths which are not infrequent, and in the early stages of conceptacles or cryptosomata. The nuclei in the thallus, except in cases of young sporelings, are generally very small and difficult to study; but to make certain as to whether the number of chromosomes remains constant in the thallus grown under normal conditions, a thorough study was made of the typical mitoses at various stages of

development. The following brief account of the essentials is illustrated by figures from the apical portions of male and female plants. Since the mitoses in male and female plants are precisely alike, the following account may be understood as applicable in either case.

The nuclei in the growing apex of the plant are somewhat larger than those in older regions of the thallus, sometimes filling almost all of the lower half of a narrow elongated palisade cell, such as constitutes the surface layer of the thallus. The cells are filled with an abundance of contents, such as plastids, physodes, and other granular substances of undetermined nature. In regions where such contents are scarce, the cytoplasm shows a very fine alveolar structure, which gradually becomes granular, even, and homogeneous toward the nuclear membrane.

In early prophase the chromatin network of the resting nucleus changes to a structure in which numerous chromatin knots become more and more pronounced, until they become transformed into well-developed chromosomes (*figs. 1a, 1b, 7*). The chromosomes, which at first appear very irregular in size and shape, now become quite similar in form, slightly bent, and in this condition they are arranged in an equatorial plate (*figs. 2, 8*).

During the chromosome development within the nucleus, the cytoplasm has a tendency to become transformed into kinoplasm at the two poles. As a rule in these kinoplasmic accumulations, centrosomes appear first in the late prophase, when the chromosomes are arranged in the equator (*fig. 2*). The nuclear membrane persists generally up to late metaphase, being especially well marked toward the equatorial region. In the polar region where the centrosomes lie, the membrane will perhaps be very faint, so as to allow the developing spindle fibers to intrude into the nuclear cavity while the astral rays are formed outside. Owing to the minuteness of the nucleus, it is rather difficult to make any positive statement in regard to the origin of the centrosome and spindle. These features can be treated more satisfactorily in connection with the divisions in the oogonium and the early segmentations of the fertilized egg.

The number of chromosomes in the early prophase (*figs. 1a, 1b*) is more than 60, but 64 chromosomes can be counted with certainty

in the late prophase (*figs. 3, 7*) and anaphase (*figs. 6, 12a, 12b*) in the polar view of the equatorial plate. In metaphase, the chromosomes at the equator split and separate, and in anaphase two sets of daughter chromosomes proceed toward the poles (*figs. 4, 10*). The sets of daughter chromosomes when they reach the poles no longer remain in one plane, but become aggregated into more or less irregular spherical masses which then become vacuolate. Probably through the interaction of the nuclear sap, derived perhaps from the vacuolation, and of the surrounding cytoplasm, a new membrane is organized, thus completing the process of typical mitosis.

The centrosomes that were always observed staining black at the poles lose gradually their sharp identity, until they can no longer be differentiated by stains.

MITOSES IN ANTHERIDIA

In Fucus antheridia develop from wall cells of the conceptacle. A wall cell of the conceptacle puts forth a papilla which is cut off by a transverse wall (*fig. 13*). The papilla grows for a time and divides, forming the antheridium and its stalk (*fig. 14*). A stalk cell may produce again either several antheridia directly or a papilla which gives rise to an antheridium and a stalk; the latter often repeats papilla formation again and again, so that there are produced conspicuous branching systems bearing numerous antheridia.

The young antheridium enlarges after its formation until its length becomes 2–4 times greater than its breadth, the growth of the cell being accompanied by that of its nucleus. The cytoplasm contains deeply staining granules and is very dense, especially in the neighborhood of the nucleus. The nucleus in the resting condition contains a comparatively large amount of chromatin substance arranged in a network evenly distributed throughout the nuclear cavity. At this time neither kinoplasmic accumulations nor centrosomes are differentiated. The nuclear network, composed of ragged chromatin, now becomes transformed into a somewhat thicker thread (*fig. 15*).

This transformation of the chromatin from a fine ragged reticulum to a thread accompanies the first manifestation of polarity in the nucleus; for it does not occur simultaneously throughout the cavity,

but is more active near the nuclear membrane. The fine ragged material previously scattered throughout the central portion of the nucleus moves toward the peripheral region and becomes transformed into chromatin threads, leaving the central part of the cavity comparatively free from chromatin (*fig. 16*). The chromatin threads become thicker and an eccentric distribution of them takes place (*fig. 17*), until finally they are grouped in synapsis at one side of the cavity (*fig. 18*).

The chromatin threads, thus eccentrically grouped in synapsis, have a certain regularity, i. e., they are not in a tangled mass or ball, but are in groups of almost parallel loops, converging to a spot where they are attached to the inside of the membrane. While this eccentric synapsis of chromatin threads is going on within the nucleus, in close association with the threads in synapsis, the cytoplasm directly outside the membrane becomes transformed into dense kinoplasm. Not infrequently there are two synaptic groups (*fig. 19*) at two opposite poles within the nucleus; and naturally in such cases two kinoplasmic accumulations appear in association with the two synaptic groups.

The loops grouped in synapsis thicken and shorten, the two arms of each loop touching each other (*figs. 20, 21*). They now condense considerably, appearing therefore a little smaller, and become detached from the spot where they lay during synapsis. Each of these condensed loops becomes a pair of bivalent chromosomes (*fig. 22*). Later, the two halves of such a bivalent chromosome become closely applied to each other, so that the whole chromosome appears to be a small spherical mass. Such is the condition of prophase.

In late prophase a single centrosome appears in the kinoplasmic accumulation at one pole (*fig. 23*), the centrosome at the other pole making its appearance later. Radiations and achromatic spindles develop in connection with the centrosomes. The spindles then attach themselves to the chromosomes and an equatorial plate is established (*fig. 24*).

It is important to note the origin of the chromatin threads or spirem in synapsis and the relation between the spirem and chromosomes. The chromatin threads in synapsis, which have arisen from the transformation of a delicate ragged chromatin reticulum in the

resting nucleus of the young antheridium, are not paired, but single. As a consequence, the loops in synapsis are single in nature. An examination of the loops cut transversely during synapsis showed that the arms of the loops are altogether about 64 in number (*fig. 21*). Finally, both ends of the loops being detached, 64 chromosomes are formed, each pair of which, being derived from two arms of a loop, becomes a pair of bivalent chromosomes. The number of these bivalent chromosomes may be readily counted again in the polar view of the equatorial plate (*fig. 25*). When the two halves of a bivalent chromosome begin to separate, the figure (*fig. 26*) shows the characteristic aspect of the heterotypic mitosis. The two sets of the daughter chromosomes then separate and proceed to the poles of the spindle (*figs. 27, 28, 29, 30*). The central spindle is of short duration; when the daughter chromosomes aggregate in a mass and organize a new nucleus, the spindle fibers entirely disappear. The centrosomes disappear at the end of telophase.

The two daughter nuclei, after a short rest, commence the second division, which is simultaneous, the antheridium remaining without much increase in size. In prophase, 32 chromosomes are differentiated from the chromatin reticulum, and in the later part of this phase two centrosomes appear (*figs. 31, 31a*) one after the other; the achromatic spindle is developed in connection with the centrosomes and an intranuclear mitotic figure is established (*figs. 32, 32a*). Metaphase (*fig. 32*), anaphase (*fig. 33*), and telophase in the two nuclei proceed simultaneously and finally four nuclei are formed.

Soon after the telophase, the cytoplasm between the four newly formed nuclei shows a fibrillar arrangement connecting the nuclei, but the display is of short duration and the four nuclei remain either in a group or scattered with no regularity along the longitudinal axis of the antheridium. The second division does not differ much from typical mitosis, except that the nuclear membrane dissolves at an earlier stage in prophase, and that no cell plate is laid down between the daughter nuclei.

The four nuclei in the antheridium, after a short rest, begin the third division, which is accompanied by a gradual growth of the cell. The third division in each of these four nuclei naturally results in eight nuclei (*figs. 34, 34a, 35, 35a, 36, 36a*). The eight nuclei give

rise by simultaneous division to sixteen nuclei (*fig. 37*). The fifth division follows at once in each of these sixteen nuclei, resulting in the formation of thirty-two nuclei (*fig. 38*). These simultaneous mitoses take place with only short resting periods between, and precisely the same as the second mitosis. The centrosome is most brilliant at the first mitosis and gradually becomes fainter in the successive divisions. The number of chromosomes in early metaphase (*figs. 34a, 37a, 38a*) and late anaphase (*fig. 36a*) of these mitoses is 32.

The formation of partition walls in the antheridium begins to take place at the 32-nucleate stage. Up to this stage, the nuclei of the antheridium are free, but finally in telophase of the fifth mitosis, with the disappearance of the central spindle in each mitotic figure, there could be seen in the neutral region between any two nuclei the faint manifestation of a protoplasmic plate formed by the transverse walls of fine alveoli becoming perceptibly thicker and arranging themselves in such a way as to appear as an uneven or somewhat zigzag line in section. The unevenly continuous walls of the alveolar lamellae grow gradually thicker, and soon uniform plates are laid down simultaneously, so that the antheridium is divided into 32 cells.

The nuclei in these cells of the antheridium undergo one more mitosis, the sixth, which results in 64 nuclei (*fig. 39*). Thirty-two chromosomes are present at this mitosis (*fig. 39a*). This last division is also accompanied by the laying down of thin protoplasmic partition walls, so that the antheridium now contains 64 cells, which are spermatids or sperm mother cells.

The nuclei in the spermatids undergo a peculiar modification, and with an accompanying change of cytoplasm surrounding the nucleus, there is produced a sperm with two cilia. The details of the events which occur in the antheridium following the 32-celled stage, as well as the development of the sperm from the spermatid, will be treated in a separate paper which will be published later.

MITOSES IN OOGONIA

It is well known that oogonia in Fucus develop from the wall cells of the conceptacle. The wall cell puts forth a papilla which divides into two cells, an oogonium and its stalk. The oogonium enlarges

to a considerable size, and three mitoses occur within, naturally producing 8 nuclei, each of which with its cytoplasm becomes an egg.

The mitoses in the oogonium of Fucus have already been studied chiefly in *Fucus platycarpus*, and in a supplementary way in *F. serratus*, by STRASBURGER, and mainly in *F. vesiculosus*, and in a supplementary way in *Ascophyllum nodosum*, by FARMER and WILLIAMS. The detailed accounts given by these authors are devoted chiefly to the last one of the three mitoses, the first and second mitoses being touched only slightly. The following is a description of the first two divisions in the young oogonium in *Fucus vesiculosus*.

The resting nucleus in the oogonium contains a delicate chromatin reticulum which is scattered irregularly throughout the cavity. The amount of chromatin substance seems rather scanty in proportion to the size of the nucleus (*fig. 40*). One or two very large nucleoli generally lie isolated in the center. The cytoplasm in general has a very delicate alveolar structure, which is very frequently interrupted here and there by plastids, physodes, and black-staining spherical bodies of undetermined nature. Toward the periphery of the nucleus, the cytoplasm assumes a mixed structure of fine granules and fibrils. The nuclear membrane seems extremely delicate. No polarity is manifested in this resting condition.

In very early prophase, a ragged chromatin reticulum gradually passes into a thread, at first branched and then becoming simple. As was described for the first mitosis in the antheridium, the transformation of the ragged chromatin into a thread is more active at the periphery of the cavity, so that after a while the chromatin threads are observed running irregularly and more abundantly along the periphery than in the center, thus leaving the center nearly free from chromatin (*figs. 41a, 41b*). When the partial distribution of the chromatin thread proceeds farther, the most of the tangled mass of threads is located at one side of the nuclear cavity (*figs. 41b, 42a, 42b*), showing the beginning of a typical synapsis.

Coordinate with these internuclear changes, kinoplasm develops and accumulates close to the nuclear membrane at a spot where it associates with the synaptic group of the threads within the nucleus. The threads gradually shorten and thicken. The irregularly tangled threads now become regularly arranged into loops. These loops

are evidently formed by the folding back repeatedly of long continuous threads, the blunt ends of which protrude toward the cavity, while the opposite ends become closely attached to the nuclear membrane (*figs. 43a, b, c; 44a, b*). The loops are therefore not independent of one another, but are connected also at the base, which is in contact with the membrane in such a way that an arm of one loop, by turning back, passes directly to the arm of the next loop. These connections of the loops at the base are detached and there results a number of loops in synapsis. The number of the loops is not easily counted from profile views; however, a section cut transversely through the loops showed that there are 64 cut ends of arms of the loops (*fig. 45*). Consequently, the number of loops is 32, each loop consisting of two arms.

The loops differ at first in their thickness and length, but by thickening and shortening they gradually become similar, a change which is more rapid in the thinner and longer ones. The loops now become more closely associated with one another during the culmination of synapsis. The two arms of each of these loops in contact with each other gradually become more compact and consequently appear smaller. The two arms of each of these loops then separate at the bend—the point of connection—and form a pair of bivalent chromosomes in prophase of the first division. The bivalent chromosomes remain for a while at the spot where they were grouped in synapsis, and then become distributed in the nuclear cavity (*fig. 46*). Therefore in Fucus, pachynema, strepsinema, and diakinesis stages are very much modified.

Some attention was paid to the centrosome. When the kinoplasmic accumulation is first visible at one pole of the nucleus in early prophase, a small body is differentiated distinctly in the midst of the accumulation, and it soon becomes surrounded by radiations. The centrosome with its radiations is always in association with the synaptic group of loops. When the bivalent chromosomes are scattered throughout the cavity (*figs. 47a, b, c*), a second centrosome makes its appearance, generally at a distance from the first one, sometimes 180° apart from it, but often not so far away (*figs. 49a, b*). The centrosome within the kinoplasm sometimes fragments into two, but then they remain side by side without separating or establishing

a new sister centrosome with new radiations. The two centrosomes in the prophase of the first division in the oogonium in Fucus seem to be entirely independent, one appearing after the other. Often the second centrosome has not yet appeared even at a late prophase, when the chromosomes are well organized (*figs. 48a, b*). The radiations seem to increase in number and elongate, probably at the expense of the cytoplasm, as the mitotic process proceeds from prophase to metaphase.

The spindle fibers at first are clearly seen developing from the area surrounding the centrosome, where the nuclear membrane seems to be so thin as to allow the intrusion of the achromatic substance. The rest of the membrane holds its contour very sharply, so that the mitotic figure is intranuclear. Thus the intranuclear spindle of Fucus seems extranuclear in origin.

In late prophase the bivalent chromosomes are arranged in the equator. The nucleolus often remains as a vacuolate structure. The axis of the figure of the first division is variable, either parallel (*fig. 50*) or perpendicular (*fig. 51*) to that of the oogonium. The nuclear membrane, as a rule, dissolves after metaphase, and yet the outline of the figure remains even to late anaphase (*fig. 53*) without the intrusion of the surrounding cytoplasm.

The number of chromosomes in prophase emerging from synapsis is 32, each being bivalent (*figs. 48a, b*). The same number is counted from the polar view of early metaphase (*fig. 52b*). In metaphase, the two halves of the bivalent chromosomes separate. These two halves are not formed by the splitting of one chromosome, but are two independent chromosomes which were two arms of one loop. Later metaphase (*fig. 53*) and anaphase (*fig. 54*) follow; in late anaphase the chromosomes near the pole are straight rod-shaped, without any apparent indication of partition (*fig. 55*). Probably the initiation of the splitting which provides for the second division may be very much delayed in this form. After telophase there are organized two daughter nuclei and the centrosomes become unrecognizable.

The second division in the oogonium follows the first after only a short rest. The differentiation of the chromosomes from the ragged chromatin reticulum and the appearance of centrosomes seem essentially the same as in the typical mitosis. The mitotic processes in

the two nuclei, from the beginning to the end, always proceed simultaneously (*figs. 56–62*). In the prophase generally a remnant of the nucleolus is seen at the side of spindle fibers (*fig. 56*) which persists to a late anaphase (*fig. 59*). The relation of the axes of the two figures varies (*fig. 57*). In early metaphase the polar view of the chromosomes in the equator shows the number to be 32 (*fig. 58b*). Anaphase and telophase follow as was described in typical mitosis. The centrosomes always persist with a beautiful display of radiations. When the daughter chromosomes reach the poles and become vacuolate, some of the central spindles seem to be replaced by fibrillar cytoplasm. The cytoplasm between the newly formed nuclei of two sister figures also changes to a fibrillar structure; thus the four nuclei are connected with cytoplasmic fibers (*fig. 63*) that resemble the late telophase of the second division of spore mother cells of some higher plants. Soon after, the fibrillar cytoplasmic structures fade entirely away, and the four daughter nuclei come into close association with one another at the center of the oogonium, and rest for a considerable period. There then follows a rapid growth in the oogonium, which almost reaches its full size before the third division begins.

Detailed descriptions of the third division were given by FARMER, WILLIAMS, and STRASBURGER, and therefore need not be repeated here. A point or two concerning chromosomes seems worth mention. In the early metaphase of the third division, when the chromosomes of a slightly bent rod-shape are arranged in the equator, they take such a position that their long axes lie parallel to the equator without overlapping one another. As a consequence, the profile view of the figure in this stage (*fig. 64*) shows the end view of the chromosomes and the polar view their whole length. It is very easy to demonstrate that there are 32 chromosomes. The chromosomes then split longitudinally in the equator (*fig. 65*), and after keeping their position (*fig. 66*) for a short time, they become directed toward the poles (*fig. 67*), and then the usual anaphase and telophase follow. Therefore the daughter nuclei contain 32 chromosomes.

FERTILIZATION AND THE FIRST SEGMENTATION DIVISION

The events which take place during fertilization as well as during the segmentation division have been described by FARMER, WILLIAMS,

and STRASBURGER. Avoiding an unnecessary repetition, a few points concerning the centrosome and chromosomes may be noted.

The resting nucleus of the discharged egg has shown no manifestation of polarity. Cytoplasmic alveolar structures as well as plastids, and spherical globules of various sizes are arranged radially about the nucleus. The cytoplasm surrounding the nuclear membrane has a finely granular aspect. When the sperm has entered the protoplast of the egg and is advancing toward the egg nucleus, a change occurs in the latter. At a certain spot outside the nuclear membrane, there is first observed a dense kinoplasmic accumulation, in which there lies a single deeply staining body very close to the membrane. Faint radiations are formed from the kinoplasm surrounding this centrosome (*figs. 68a, 68b*). The egg nucleus, therefore, is furnished with a single centrosome before the sperm reaches it. The second centrosome has been found to appear in connection with the sperm.

While the sperm is proceeding toward the nucleus, there appear numerous irregularly crowded granules, surrounding the nuclear membrane. The size of these granules at first is not very different from that of the centrosome of earlier occurrence, but they rapidly grow larger and are either spherical or (sometimes) elongated. Their growth, thus, is different from that of the centrosome, so that small granules of the same size as young centrosomes can be distinguished from genuine centrosomes. Such is the condition of the region surrounding the nucleus just before the appearance of the second centrosome. The sperm then reaches the egg nucleus, becomes closely applied to it, and seems to slip in through the nuclear membrane (*fig. 69b*). At this very instant, there is first observed a new centrosome with radiations, appearing at the spot where the sperm entered.

The second centrosome might have been brought in some way by the sperm, as was suggested by STRASBURGER (**24**). Or it is probable that one of the granules surrounding the nucleus might have been brought to the spot mechanically by the streaming movement of kinoplasm caused by the progress of the sperm. At any rate two centrosomes do appear, one after the other, the first one being visible before the entrance of the sperm, and the second arising in connection with the entry. That the appearance of the second

centrosome is always associated with the sperm is evidenced by cases of polyspermy (*figs. 76–79*).

Coalescence of the egg and sperm, the entry and progress of the sperm in the egg cytoplasm, and the entry of the sperm into the nucleus, all occur with rapidity. The chromatin of both the sperm and egg nuclei forms the reticulum of the fusion nucleus. The chromatin of both nuclei is mingled so as to become indistinguishable (*figs. 71a, b, c*). The mitoses at the segmentation of normally fertilized eggs (*figs. 72a, b; 73; 74; 75a, b, c*) take place as described by STRASBURGER and by FARMER and WILLIAMS. The number of the chromosomes in the prophase is 64 (*fig. 75a*).

In cases of polyspermy, when two sperms enter the egg nucleus, two centrosomes appear in the two spots where the sperms entered; when three sperms have entered, there are three centrosomes. In case of bispermy there are developed three poles, and in case of trispermy (*fig. 76*) four poles (*fig. 77*) are present; for one pole has already appeared before the sperm enters. In the nucleus with three poles, there are tripolar spindles, and 96 chromosomes become distributed upon the three spindles. The chromosomes split longitudinally at the metaphase, and at telophase two sets of 32 chromosomes meet at each of the three poles to form three daughter nuclei.

In a quadripolar spindle (*fig. 78*) 128 chromosomes are distributed upon six spindles, and each of the four poles receives three sets of daughter chromosomes, numbering 21, 21, and 22 (*fig. 79b*), to form daughter nuclei. In cases of polyspermy, the formation of daughter nuclei occurs simultaneously.

It is very interesting to note that in these cases of polyspermy, the constancy of the number of the chromosomes is maintained by producing multipolar spindles. Whether or not polyspermy may occur in natural conditions has not been determined.

DISCUSSION OF CYTOLOGICAL PHENOMENA

The problem of cilia-bearing structures and centrosomes and their possible relationship is treated best in such a form as Fucus, in which both blepharoplasts and centrosomes are present. As the problem is quite important, it will be treated in detail in the next paper. At present only a brief account of the chromosomes will be given.

Origin of the bivalent chromosomes.—Although the actual segmentation of the chromosomes in Fucus occurs just after the nucleus has emerged from synapsis, their virtual preformation, as continuous chromatin threads from which the chromosomes develop, begins very early in prophase. As was described before, the ragged reticulum of chromatin in the resting nucleus gradually becomes transformed into a thread running in various directions, the transformation being very much more active at the periphery than in the center of the nuclear cavity. The threads in their beginning are uneven and branched, then they become much evener and the transformation continues, so that long continuous threads are formed, running mostly in the peripheral region of the cavity. The threads thus formed seem to have no ends (*fig. 42*), and apparently form one continuous thread. Moreover, any part of the thread shows its single nature from the early beginning of the transformation up to its completion as a continuous structure. Entering into the synaptic condition, the single thread then shortens and thickens, and becomes eccentrically grouped as a loose tangled mass at one side of the nuclear cavity; so that eventually a number of loops are formed by the repeated folding of the thread (*figs. 18, 20, 43, 44*). The loops so developed are therefore still continuous with one another at the bases where they come in contact with the nuclear wall. The loops then become arranged in a loose bunch, parallel and regular, with their bases attached to the nuclear membrane, while the opposite folds protrude into the cavity. Then the loops continue to shorten and thicken and become more and more aggregated; each loop then folds at its bent end so that the bent arms are in contact with each other, when synapsis has reached its culmination. As they emerge from synapsis (*fig. 46*), there are present 32 bivalent chromosomes, which become detached from the nuclear membrane, moving toward the various regions of the nuclear cavity.

The relationship of the chromatin thread in prophase, the loops in synapsis, and the bivalent chromosomes of postsynapsis, may be clearly followed. A pair of bivalent chromosomes corresponds to one of the loops in synapsis; the loops being formed by a folding-back of the chromatin thread, so that a loop in synapsis should be considered as composed of two sporophytic chromosomes arranged end

to end. If we apply a modern interpretation of synapsis to this case of Fucus, the chromatin of paternal and maternal origin becomes arranged in early prophase, not in parallel threads, but with the chromosomes end to end, so as to form a single thread, which, passing the so-called leptonema stage, enters into the synaptic condition, during which there probably takes place a close association of the chromatin of the two origins. In this case, the pachynema and strepsinema stages (if they occur at all) must be of very short duration, and consequently the chromatin thread of the zygonema condition in synapsis passes directly into the diakinesis stage. The two elements of the bivalent chromosomes then separate from each other, thus effecting what may be regarded as a reduction. Generally in Fucus the initiation of the longitudinal splitting which provides for the second division does not occur even in late anaphase of the first division, but probably may occur before the organization of the daughter nuclei, as in the generally accepted account of sporogenesis.

Neglecting for a moment the many points which differ in particulars, the results in Fucus, namely, that the chromosomes emerging from synapsis show the reduced number, and that the reduction has taken place by an end-to-end fusion of sporophytic chromosomes, agree in essentials with the views published by FARMER and MOORE (**6, 7**), SCHAFFNER (**21, 22**), MOTTIER (**15, 16**), and STRASBURGER (**25**), and by one group of zoologists, such as VOM RATH (**17**), RÜCKERT (**20**), and MONTGOMERY (**13, 14**).

Regarding the origin of bivalent chromosomes, however, the author is fully convinced of the correctness of the interpretation that in the majority of cases now investigated, two independent threads originate in early prophase and become associated side by side in synapsis, and that when the two threads emerge from synapsis they form the two elements of the bivalent chromosome. Such cases were clearly established by GRÉGOIRE (**10, 11, 12**), BERGHS (**3, 4**), ALLEN (**1, 2**), ROSENBERG (**18, 19**), and some others, including the author himself (**30, 31**). The author, in a forthcoming paper on sporogenesis in *Osmunda cinnamomea* has reached the same conclusion as has the latter group of investigators. The results in Fucus, however, are not deniable. It is not inconceivable that there are two distinct types of arrangement of sporophytic chromosomes at synapsis.

Constancy in the number of chromosomes.—After the appearance of STRASBURGER's classical paper (**23**) on "Periodic reduction of the number of chromosomes in the life-history of living organisms," investigators of many forms added to the evidence in favor of the proposed theory. A plant is known to have a certain number of chromosomes, without much variability, in one phase of its life-history When the number is not too great, an accurate counting is not difficult. The larger the number, however, the more difficult the counting becomes, especially when the chromosomes are long and filamentous, because the stages favorable for exact counting then become more and more narrowly limited.

Unfortunately the rarity of the favorable stages has led some investigators to the hasty conclusion that the counting is almost impossible, while others, being unable to find the favorable stage, have tried to make a rough estimate of the number from such stages as they had. It is no wonder that such rough estimates, based upon stages unfavorable for counting, should vary. It is curious to note that even in *Nephrodium molle*, which contains 66 chromosomes in the gametophyte and 132 in the sporophyte, the number was clearly counted by the author both in apogamous and in normal forms, while FARMER and DIGBY (**5**) claimed that the number of chromosomes varied in the allied forms of *Nephrodium molle* which they studied. The constancy in the number of chromosomes in normal cases has been cited as one of the important proofs of the individuality of the chromosome, and the importance of this theory in any discussion of heredity cannot be neglected.

In the present investigation of *Fucus vesiculosus*, the number of chromosomes was counted in mitoses in the vegetative cells of male and female plants, in the antheridium, in the oogonium, and in sporelings. In the vegetative cells, from the polar view of both early metaphase (*figs. 3, 9*) and anaphase (*figs. 6; 12a, b*), 64 chromosomes were counted. Although the antheridium is very small, the polar view of the mitotic figures in early metaphase showed clearly the same number, as 32 bivalent chromosomes in the first division and 32 univalent chromosomes in the mitoses following the second division. In the first mitosis in the oogonium 32 bivalent chromosomes are present (*figs. 47, 48, 52*) and, as in the antheridium, 32 univalent ones

appear in the second (*fig. 58*) and third (*fig. 64*) mitoses. In the first division of the fertilized egg there are 64 chromosomes arranged in the equator. Thus, the number is constantly 32 and 64 in *Fucus vesiculosus*.

FARMER and WILLIAMS (**9**) state that in *Ascophyllum nodosum* the approximate number estimated in the mitotic figure of the oogonium mother cell is about 26–30, and later on they counted in the third division in the oogonium 14–15 as the reduced number. STRASBURGER (**24**) considers 30 to be the probable number in *Fucus platycarpus*, in which he studied chiefly the division in the oogonium. Such a difference in the number of chromosomes in different species of the same genus or in allied forms which grow in normal conditions has also been known in other cases; for instance, in Osmunda, where *Osmunda regalis* has 12 and 24 chromosomes and *O. cinnamomea* 22 and 44.

Alternation of generations.—It has been suggested by STRASBURGER (**26**) that the antheridia and oogonia in young stages (*Anlagen der Oogonien und Antheridien*) should be regarded as corresponding not with antheridia and oogonia of Dictyota but with its tetrasporangia, although the exact phenomena of reduction which occurs in the first two divisions in these structures was not then known in detail. The present result may confirm the correctness of his suggestions.

Briefly summarizing the nuclear conditions of Fucus: The vegetative cells of the plant contain 64 chromosomes, and the same number is present up to the formation of antheridium and oogonium initials. In the first nuclear division in these initials 32 chromosomes appear, the reduced number, but they are bivalent. At the telophase of the second division there are 32 univalent chromosmes. Consequently, the four nuclei resulting from the second division in both oogonium and antheridium initials are the first nuclei which contain 32 univalent chromosomes. Each of the four nuclei divides further within these structures, once in the oogonium and four times in the antheridium, and after the division or divisions there result 8 egg nuclei or 64 sperm nuclei, each nucleus containing 32 chromosomes. At the union of the sperm and egg nuclei, the number is doubled, and the sporeling with the diploid number of chromosomes develops into a Fucus plant.

It would follow that the antheridium and oogonium initials up to the second division may be well compared with spore mother cells in the higher plants, and that the four nuclei in these structures thus produced may be compared with microspores and megaspores, which in Fucus germinate at once within the oogonia and antheridia, and the gametophyte generations, thus initiated, undergo only one mitosis in the oogonium and four mitoses in the antheridium. Thus in Fucus the gametophyte generation with the haploid number extends from the tetranucleate stage both in antheridium and oogonium initials, up to the formation of the sperm and egg. With the union of the gametes, the sporophyte generation with the diploid number of chromosomes begins, and it terminates with the development of the four nuclei in the antheridium and oogonium initials.

SUMMARY

The nuclear conditions during the life-history of *Fucus vesiculosus* may be summarized as follows:

1. The Fucus plant contains 64 chromosomes and the number is reduced at the end of the first two nuclear divisions in the oogonium and antheridium initials.

2. Each of the four nuclei produced at the end of the first two divisions contains 32 univalent chromosomes, and this number persists up to the formation of the sperm and egg; the phase containing 32 chromosomes may be regarded as the gametophyte generation.

3. The union of the gametes doubles the number, and 64 chromosomes are present in every mitosis through the development of the Fucus plant up to the formation of the first four nuclei in the oogonium and antheridium initials. The phase containing 64 chromosomes may be regarded as the sporophyte generation.

4. There is thus present in Fucus an alternation of the gametophyte generation containing 32 chromosomes, with the sporophyte generation containing 64 chromosomes.

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EXPLANATION OF PLATES VIII–XI

The figures were drawn with the aid of an Abbé camera lucida, under Zeiss apochromatic obj. 1.5^{mm} N. A. 1.30, with compensating ocular 12; except *figs. 1–12, 15–29, 31a, 32a, 34a, 35, 36a, 37a, 38a, 39a,* which were drawn with compensating ocular 18; *figs. 13, 14, 30, 31, 32, 33, 34, 36, 37, 38, 39, 76–79,* drawn with ocular 8; and *figs. 42, 48, 50, 51, 53, 56, 57, 59, 60, 63,* drawn with ocular 4. The plates are reduced to one-half the orginal size.

PLATE VIII

Mitoses in the vegetative cells of the male plant

FIGS. *1a, 1b.*—Two sections of the same nucleus in the cortical layer of the thallus; no centrosome has appeared, the nucleus is in early prophase and the approximate number of chromosomes can be estimated from the two sections as near 64.

FIG. 2.—Late prophase: two poles; centrosomes in the center.

FIG. 3.—Stage similar to *fig. 2,* viewed from pole: chromosomes 64.

Fig. 4.—Metaphase: two sets of daughter chromosomes separated.

FIG. 5.—Anaphase: each of the two sets of daughter chromosomes arranged almost in a plane.

FIG. 6.—Stage similar to *fig. 5*, viewed from pole: chromosomes 64.

Mitoses in the vegetative cells of the female plant

FIG. 7.—Early prophase of the nucleus in vegetative cells of the thallus: chromosomes (estimated) about 64.

FIG. 8.—Early metaphase: centrosomes with a few radiations.

FIG. 9.—Stage similar to *fig. 8:* chromosomes 64.

FIG. 10.—Anaphase.

FIG. 11.—Late anaphase.

FIGS. 12*a*, 12*b*.—The same stage as *fig. 11*, viewed from pole: two sets of 64 chromosomes.

Mitoses in antheridia

FIG. 13.—Developing papilla, to become later an antheridium: nucleus approaching prophase.

FIG. 14.—Later stage of papilla: nucleus in anaphase; when this mitosis is completed, there will be formed a stalk cell and an antheridium.

FIG. 15.—Nucleus of the antheridium in resting condition, showing delicate chromatin network: no centrosome.

FIG. 16.—Nucleus with chromatin network beginning to be transformed into a more or less pronounced thread structure: nucleolus without any connection with the network; no centrosome.

FIG. 17.—Nucleus with first indication of polarity: chromatin thread more thickly tangled at one corner of the nuclear cavity; cytoplasm begins to show kinoplasmic nature.

FIG. 18.—Nucleus in synapsis: parallel chromatin loops protrude from one side of nuclear membrane into the nuclear cavity.

FIG. 19.—Nucleus in synapsis: most of the chromatin loops aggregated at two poles; a few threads traverse the nuclear cavity, connecting the poles; this case is rare.

FIG. 20.—Nucleus still in synapsis: the loops thickened and shortened.

FIG. 21.—The same stage, viewed at right angles.

FIG. 22.—Early prophase just after synapsis: chromosomes showing bivalent nature.

FIG. 23.—Prophase: a centrosome at one pole; the two constituents of the bivalent chromosome come in close contact, so its double nature cannot be recognized.

FIG. 24.—Metaphase: a number of chromosomes arranged in the equatorial plate; centrosomes with radiations.

FIG. 25.—The same stage as *fig. 24*, viewed from the pole, showing 32 (in reality bivalent) chromosomes.

FIG. 26.—Late metaphase: bivalent chromosomes in the equatorial plate at the point of separation, revealing characteristic feature of heterotypic mitosis.

FIG. 27.—Anaphase: two sets of daughter chromosomes proceeding toward the poles.

FIG. 28.—Late anaphase.

FIG. 29.—Telophase of the first (heterotypic) division in the antheridium: centrosomes faintly discernible.

FIG. 30.—The antheridium after the first nuclear division: two daughter nuclei in the resting condition; no centrosome.

FIG. 31.—Prophase of second mitosis in the antheridium: two daughter nuclei in similar stage; centrosomes present.

FIG. 31*a*.—One of the two nuclei shown in *fig. 31*, under higher magnification: chromosomes 32.

FIG. 32.—Metaphase: two figures in the same condition.

FIG. 32*a*.—One of the two nuclei shown in *fig. 32*, under higher magnification.

FIG. 33.—Late anaphase: mitosis proceeding simultaneously in the two nuclei.

FIG. 34.—Late prophase of the third nuclear division in the antheridium: four figures in similar condition.

FIG. 34*a*.—One of the four nuclei shown in *fig. 34*, under higher magnification.

FIG. 35.—Metaphase, viewed from the pole: each of the 32 chromosomes has just split.

FIG. 36.—Anaphase: the four nuclei in the same condition.

FIG. 36*a*.—One set of daughter chromosomes from *fig. 36*, under higher magnification: chromosomes 32.

FIG. 37.—Late prophase of the fourth mitosis in the antheridium: eight figures in the same stage.

FIG. 37*a*.—One nucleus in late prophase from *fig. 37*, under higher magnification: chromosomes 32.

FIG. 38.—Late prophase of the fifth mitosis in the antheridium: sixteen figures in similar condition.

FIG. 38*a*.—One nucleus from *fig. 38*, under higher magnification: chromosomes 32.

FIG. 39.—Late prophase of the sixth nuclear division in the antheridium: thirty-two figures in the same stage.

FIG. 39*a*.—One nucleus from *fig. 39*, under higher magnification: chromosomes 32.

PLATE IX

Mitoses in oogonium

FIG. 40.—Resting nucleus of the oogonium: chromatin showing ragged structure and nucleolus without apparent connection with it; no centrosome.

FIGS. 41*a*, 41*b*.—Two sections of the same nucleus in very early prophase: ragged chromatin transformed into a thread; a centrosome has made its appearance with a few radiations.

FIG. 42.—Early stage of synapsis: centrosome with radiations not shown in this figure.

FIG. 42*a*.—Nucleus from *fig. 42*, under higher magnification: chromatin threads very much tangled; centrosome not shown here.

FIGS. 43*a*, 43*b*, 43*c*.—Three sections of the same nucleus in synapsis: chromatin threads in form of loops becoming attached by their ends to a part of the nuclear membrane, outside of which there lies a centrosome with radiations.

FIGS. 44*a*, 44*b*.—Two sections of the same nucleus in synapsis, similar stage to above: there a black staining body associated with a nucleolus.

FIG. 45.—Section through the base of crowded loops, at contact with the nuclear membrane, showing 60 or more isolated chromatin dots, some of them connecting with one another; the dots are either the ends of the loops or their optical sections.

FIG. 46.—Nucleus emerging from synapsis: chromatin loops moving from the place of aggregation in synapsis; two arms of each of these loops are always in close association, forming bivalent chromosomes; centrosome in next section.

FIGS. 47*a*, 47*b*, 47*c*.—Three sections of the same nucleus in prophase: 32 bivalent chromosomes; now two centrosomes lie at two poles, one of the centrosomes being newly formed, independent of the other that appeared at a previous stage; some of spindle fibers beginning to intrude into the nuclear cavity.

FIGS. 48*a*, 48*b*.—Two sections of the same oogonium: 32 bivalent chromosomes; these figures show the case where there is still only one centrosome.

FIGS. 49*a*, 49*b*.—Two sections of the same nucleus in prophase: two poles less than 180° apart; intruding fibers attaching to chromosomes.

FIG. 50.—Early metaphase: intranuclear figure established, its axis parallel to that of oogonium.

FIG. 50*a*.—Intranuclear figure in prophase from *fig. 50*, under higher magnification: remnant of nucleolus still visible near the spindle.

PLATE X

FIG. 51.—Metaphase a little later than the stage in *fig. 50*, with the axis of the figure at right angles to that of the previous one.

FIG. 51*a*.—Nucleus from *fig. 51*, under higher magnification: the nuclear membrane has disappeared.

FIGS. 52*a*, 52*b*, 52*c*.—Three sections of the same nucleus in metaphase: the middle section shows 32 bivalent chromosomes, although their bivalent nature is hardly discernible.

FIG. 53.—Anaphase: the case where the contour of the nucleus still remains undisturbed even after the dissolution of its membrane.

FIG. 54.—Nucleus in anaphase, similar stage to *fig. 53*, under higher magnification.

FIG. 55.—Portion of one set of daughter chromosomes in late anaphase, showing their rod-shape while attached to the spindle fibers.

FIG. 56.—Prophase of the second mitosis in the oogonium: two figures similar.

FIG. 56*a*.—Nucleus from *fig. 56*, under higher magnification: the figure is intranuclear; nucleolus still remains.

FIG. 57.—Oogonium in which two nuclei show early metaphase: two figures perpendicular to each other.

FIG. 57*a*.—One of two figures in *fig. 57*, under higher magnification.

FIGS. 58*a*, 58*b*, 58*c*.—Three sections of the same nucleus in early metaphase: the middle one shows 32 univalent chromosomes in the equatorial plate.

FIG. 59.—Oogonium with two nuclei in early anaphase.

FIG. 59*a*.—Nucleus from *fig. 59*, under higher magnification: nucleolus still remains; two centrosomes still showing conspicuous radiations.

FIG. 60.—Oogonium with two nuclei in late anaphase.

FIG. 60*a*.—Nucleus from *fig. 60*, under higher magnification.

FIG. 61.—Telophase: two centrosomes with their radiations still recognizable; central spindle about to disappear.

FIG. 62.—Late telophase: chromosomes aggregated at poles beginning to vacuolize; meshes of cytoplasm arranged somewhat radially from two poles toward the equator.

FIG. 63.—Section of oogonium cut transversely through its axis, after late telophase of second mitosis: only three of four daughter nuclei are figured; between every two of these three nuclei is an irregular fibrillar arrangement of cytoplasm.

FIG. 64.—Late prophase of the third division, viewed from pole: chromosomes (32) in the equatorial plate before splitting.

FIG. 65.—Metaphase: nuclear membrane still present; most of the chromosomes arranged in the equator show their ends, the stage being just after splitting.

FIG. 66.—Late metaphase: nuclear membrane almost dissolved; daughter chromosomes beginning to separate.

FIG. 67.—Anaphase: nuclear membrane has disappeared, the contour of the spindle-shaped nucleus undisturbed.

PLATE XI

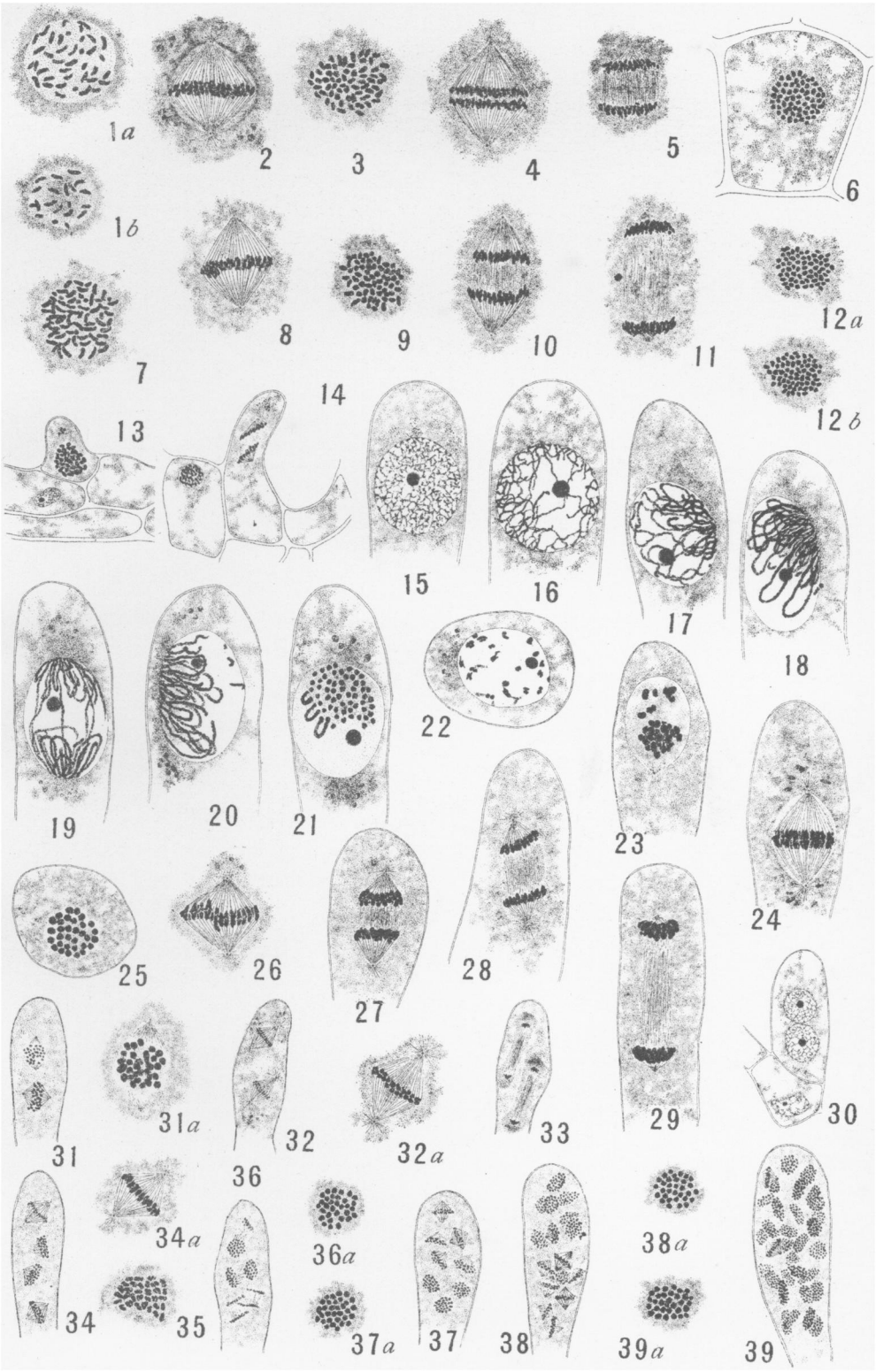
Fertilization and segmentation of fertilized egg

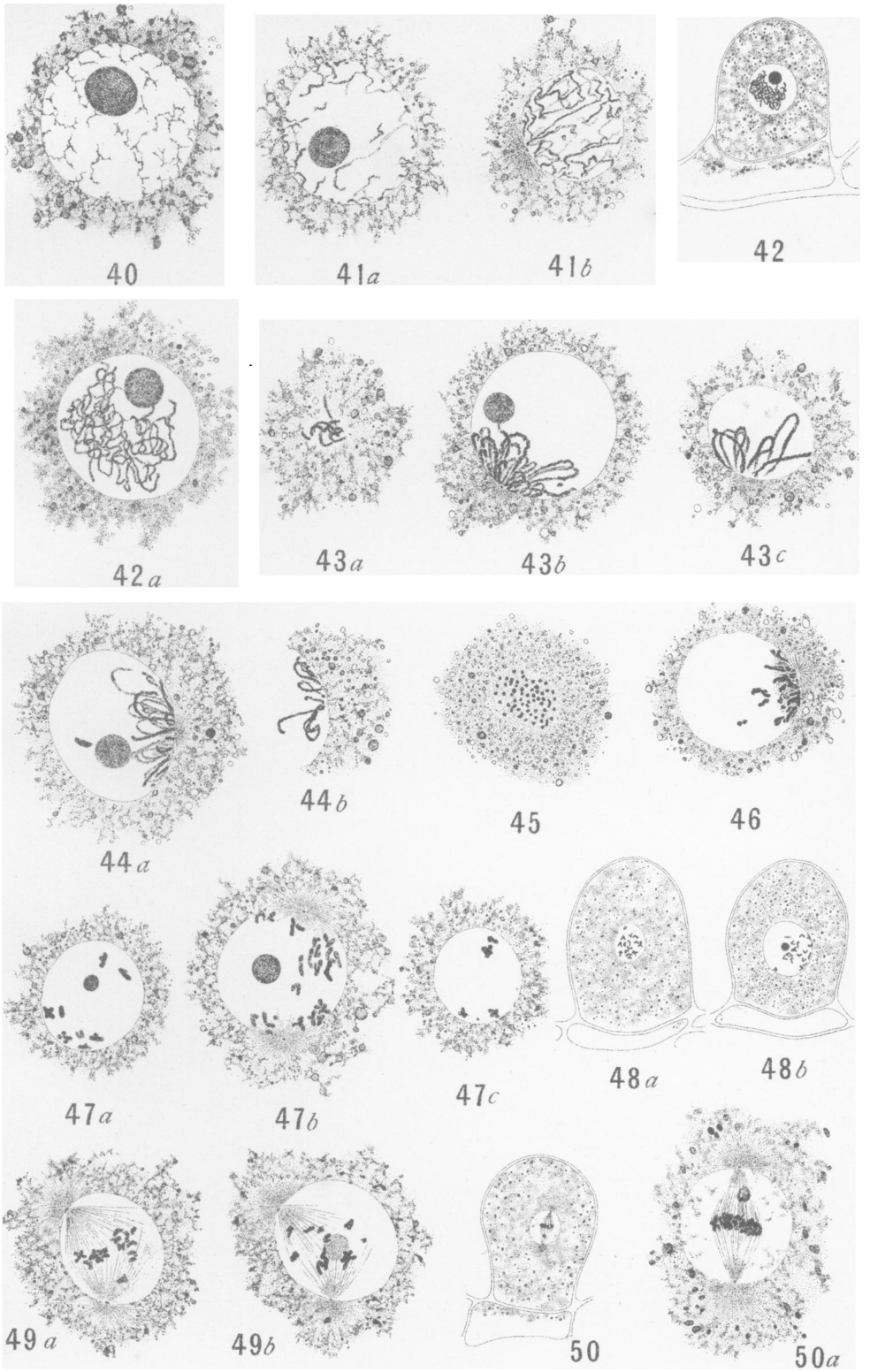
FIGS. 68*a*, 68*b*.—Two sections of the same nucleus in resting condition, from a discharged egg before fertilization, showing delicate ragged chromatin and two nucleoli; a single centrosome close to the nuclear membrane, without any radiations (*fig. 68b*).

FIGS. 69*a*, 69*b*.—Two sections of the same nucleus of an egg when a sperm nucleus has just coalesced with it; *fig. 69b* shows a new centrosome which has appeared at the point of the nuclear membrane where the sperm entered; the other centrosome, of earlier appearance, shown in *fig. 69a*, seems split into two by this time; numerous granules surrounding the nuclear membrane stain deeply.

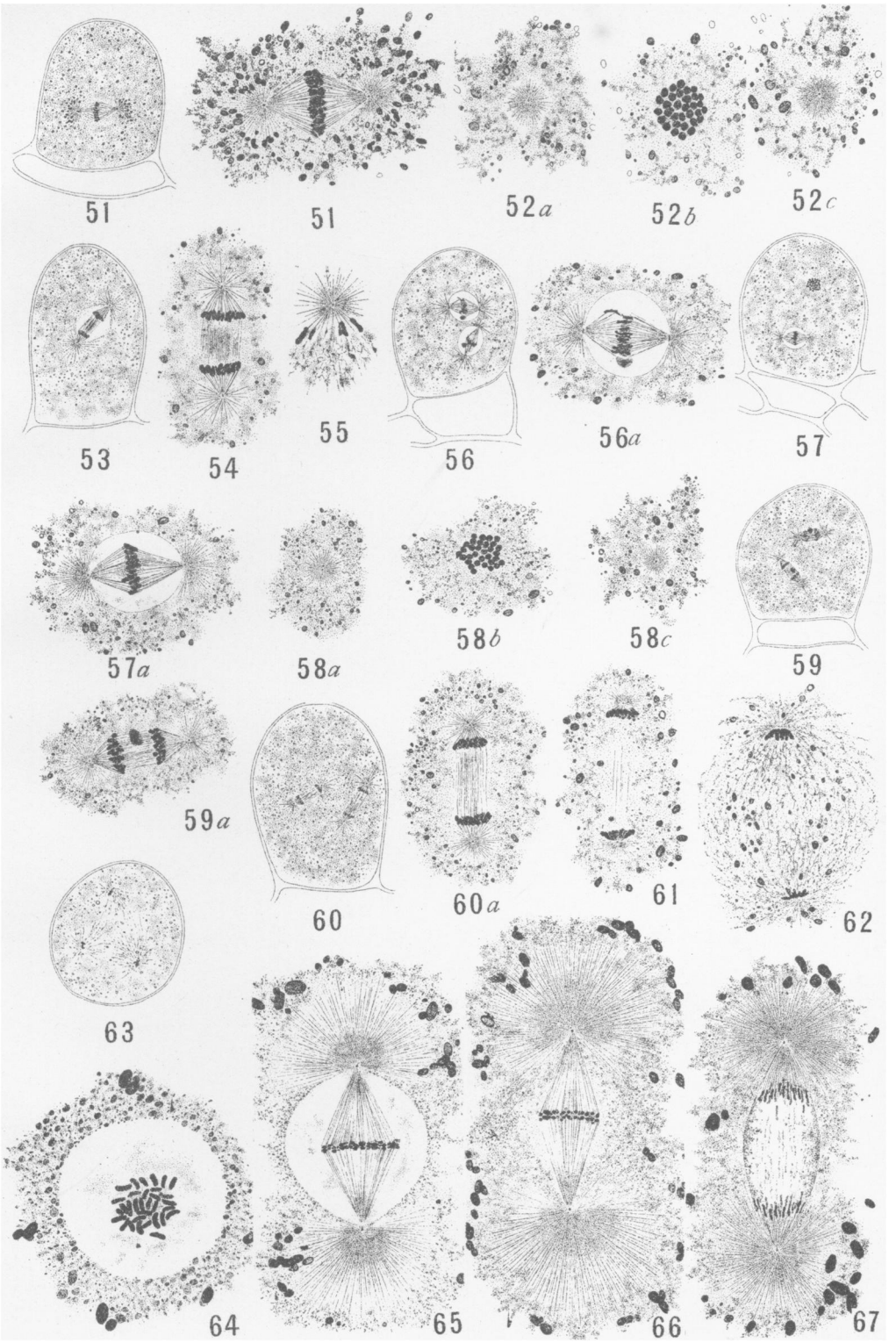
FIGS. 70*a*, 70*b*.—Two sections of the same nucleus of an egg after coalescence with sperm nucleus, a little later stage than *fig. 69*. The sperm nucleus has begun to disintegrate, a delicate chromatin reticulum of the egg nucleus still remaining in the resting condition.

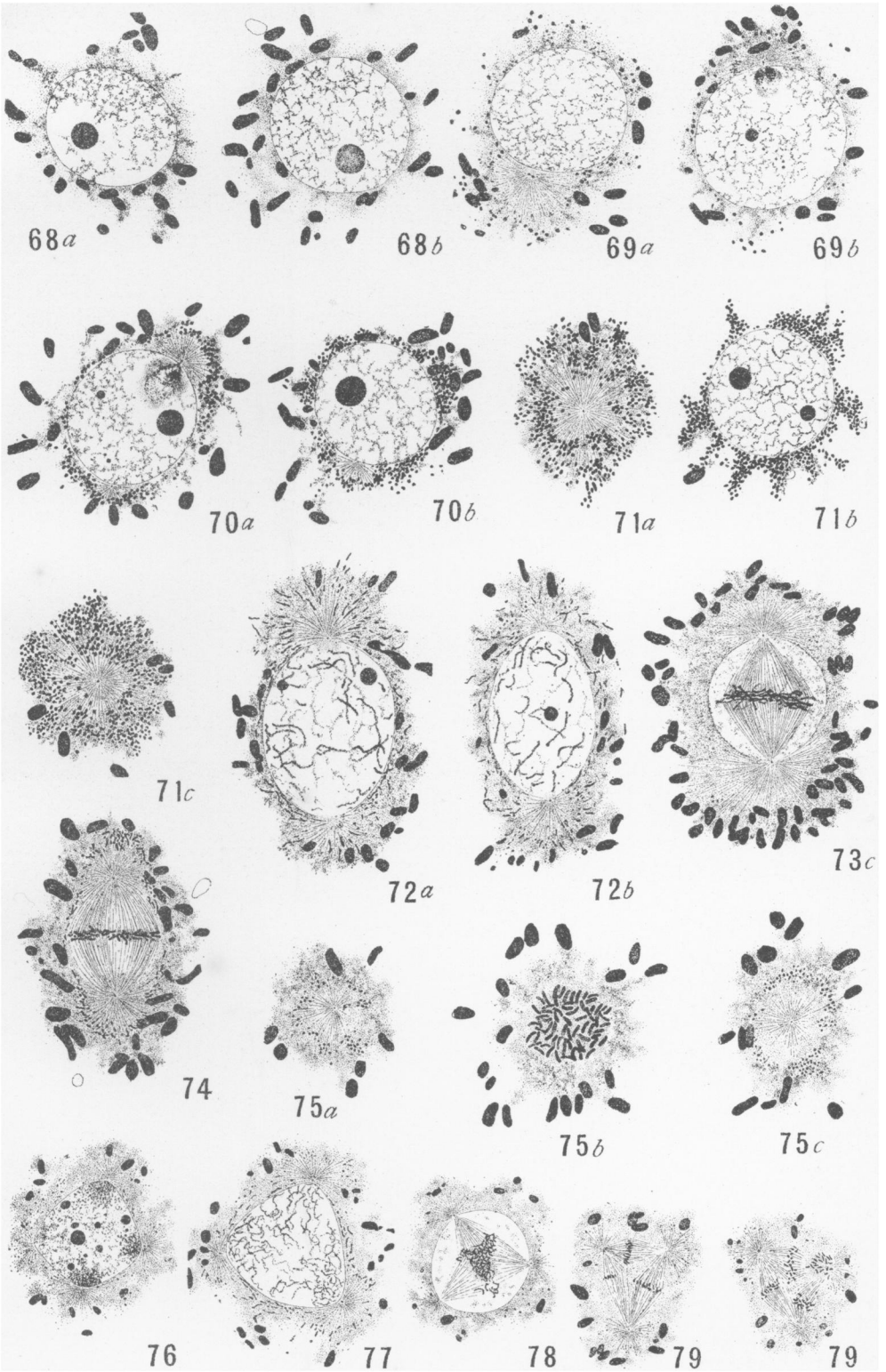
FIGS. 71*a*, 71*b*, 71*c*.—Three sections of the same fusion nucleus: the disintegrating sperm nucleus has completely mingled with the contents of egg nucleus,





YAMANOUCHI on FUCUS





so that there is now a homogeneous chromatin reticulum throughout the whole cavity of the fusion nucleus.

FIGS. *72a*, *72b*.—Early prophase of the first division in the fusion nucleus: parts of chromatin threads begin to become pronounced, suggesting prochromosomes.

FIG. 73.—Late prophase.

FIG. 74.—Early metaphase: nuclear membrane has disappeared; chromosomes not yet split.

FIGS. *75a*, *75b*, *75c*.—Same stage as *fig. 74*, cut perpendicular to the axis of the spindle: *fig. 75b* shows polar view of 64 chromosomes arranged in the equator.

Cases of polyspermy

FIG. 76.—Nucleus of fertilized egg with three sperms: a centrosome with radiations started in connection with each of the spots where the sperms entered.

FIG. 77.—Early prophase: one of the four poles not shown.

FIG. 78.—Prophase, showing quadripolar spindle: one of the four poles not shown.

FIGS. *79a*, *79b*.—Two sections of the nucleus in early metaphase; six equatorial plates shown from various views, one of them showing a polar view with 21 chromosomes.