CHROMOSOMAL ALTERNATION OF GENERATIONS IN NEREOCYSTIS LUETKEANA
(Mertens) Postels and Ruprecht

by

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ABSTRACT

A cytological examination of the life-history of Nereocystis luetkeana has shown that an alternating chromosome number corresponds to the morphological alternation of generations. The first division sequence of the zoosporangial nucleus is meiotic and is followed by three mitotic divisions. The result is a mature sporangium containing 32 nuclei. Thirty-two zoospores are liberated from each sporangium and their germination gives rise to male and female gametophytes. Genotypic determination of the sexes is believed to take place in Nereocystis. Mitosis in the gametophytes is regular and cytokinesis follows each nuclear division, producing few cells in the female and many cells in the male gametophytes. Thirty-one chromosomes can be counted at the mitotic prophase. Oogamy exists in Nereocystis and fertilization takes place after the egg is extruded from the oogonium. The sporophyte develops initially into a uniseriate filament of 5 - 8 cells before divisions in a second plane give rise to a flat, monostromatic thallus. Nuclear division in the sporophyte appears to be preceded by division of the nucleolus. Colorless and non-septate rhizoids develop as elongations of the basal cells of the sporophyte.

Some of the unfertilized eggs develop parthenogenetically and give rise to stunted, deformed plants with multinucleate cells. Temperature is an important factor in the development of various stages of the life cycle of Nereocystis grown in culture. This is particularly evident in the gametophytic stage where sexual structures are produced only at temperatures less than 10° C. and vegetative growth is most prolific at 14 - 18° C.

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(Mertens) Postels and Ruprecht

A INTRODUCTION

I General Survey

In 1928 Hartge established that Nereocystis luetkeana, grown in culture, underwent a morphological alternation of generations typical of the order Laminariales to which it belongs. She did not observe fertilization nor did she provide any cytological basis for the alternation of generations. Cytological elucidation has been given for various members of the Laminariales (Table 1), and fertilization has been examined cytologically for Pterygophora californica (McKay, 1933) and Eisenia arborea (Hollenberg, 1939). Since neither the cytological details nor the fertilization process have been observed in Nereocystis luetkeana, the present investigation was undertaken to delimit cytologically the haploid and diploid phases of the life cycle. Particular attention was paid to the mitotic and meiotic division sequences and the fertilization process was examined in some detail.

The present status of the knowledge concerning the cytology of the Laminariales is presented in a review of the pertinent literature. Kylin (1918) working with Chorda filum was the first to observe the meiotic process in the Laminariales. He found that reduction division took place in the first division of the zoosporangial nucleus. Work of this nature was not repeated until 1928, when Myers observed meiosis in Egregia menziesii. Meiosis has since been examined in the sporangia of various Laminariales and chromosome counts have been recorded (Table 1).

There is general agreement among the various workers regarding the main division sequence of meiosis within the sporangium of the Laminariales.

The interphase or resting nucleus possesses a distinct nucleolus and a very fine reticulum, although it was reported to be granular in Undaria pinnatifida (Inoh and Nishibayashi, 1954), and was figured as being granular in Laminaria japonica (Abe, 1939). Prophase is a typically extended phase commencing with synizesis, which is followed by a "spireme" stage. Synapsis occurs early in prophase (Kylin, 1918; Yabu, 1956) after which the synapsed units condense to the diakinesis stage. During this stage O-, Y-, V-shaped and parallel configurations of the bivalent chromosomes were reported in Pterygophora californica (McKay, 1933), Laminaria japonica (Abe, 1939), Undaria pinnatifida (Inoh and Nishibayashi, 1954), and Laminaria angustata (Nishibayashi and Inoh, 1956). The nucleolus and nuclear membrane have usually disappeared by diakinesis; however, the nuclear membrane has been reported to remain in Costaria costata, until the chromosomes have become oriented at the central region (Nishibayashi and Inoh, 1957). Metaphase develops with an aggregation of the chromosomes at the center of the cell and the spindle apparatus and centrosomes appear. Centrosomes were not observed at any time during the division cycle in Egregia menziesii (Myers, 1928), Eisenia arborea (Hollenberg, 1939) and Laminaria flexicaulis and Laminaria saccharina (Mange, 1953). Anaphase is a uniform separation of two groups of closely associated chromosomes. At telophase the nuclear membrane and nucleolus reappear and the chromosomes revert to interphase. Since subsequent divisions show the reduced number of chromosomes the first division is reductional. In most of the Laminariales there are usually four additional divisions resulting in a total of 32 nuclei, each of which together with a chromatophore and some sporangial cytoplasm develops into a zoospore. Occasionally 64 zoospores were observed in Pterygophora californica (McKay, 1933) and Eisenia arborea

(Hollenberg, 1939). Sauvageau (1918) counted 128 zoospores in Sacchorhiza bulbosa. The zoospores contain a nucleus, a single chromatophore, an eyespot, and possess 2 flagella. No mention of an eyespot is made for Laminaria flexicaulis and Laminaria saccharina (Mange, 1953), Undaria pinnatifida (Inoh and Nishibayashi, 1956), Alaria crassifolia (Yabu, 1956), and Costaria costata (Nishibayashi and Inoh, 1957). Presumably this is due to the fact that the above authors were not working with living material. Chromatophore development was not reported for Laminaria flexicaulis and Laminaria saccharina (Mange, 1953).

In instances where zoospores of the Laminariales have been observed, they were found to be pyriform and to possess two unequal flagella inserted laterally. The longer flagellum projects anteriorly and is two or three times as long as the zoospore; the shorter one projects posteriorly and is approximately the length of the zoospore. In culture, the zoospores remain active for a short period and then attach to the substrate, lose their flagella, round up, and develop a rigid wall. The flagella are reported to be withdrawn into the zoospore in Pterygophora californica (McKay, 1933). Spore germination begins with the development of a tubular outgrowth into which the spore nucleus migrates along with the chromatophore and the cytoplasm. A wall forms separating the new cell (tube-cell) from the spore, which is usually devoid of all stainable material. However, nuclear division has been reported to occur before the wall forms in Laminaria flexicaulis and Laminaria saccharina (Sauvageau, 1916a), Alaria esculenta (Sauvageau, 1916b), Chorda and Laminaria (Williams, 1921), Pterygophora californica (McKay, 1933) and Alaria and Laminaria (Cole, personal communication). In such instances the nucleus remaining in the spore case usually degenerates, although Sauvageau has

indicated that the spore may give rise to new cells. Repeated division of the tube-cell gives rise to a more or less extensively branched male or female gametophyte. The sexes have been proven to be genotypically determined in Laminaria saccharina (Schreiber, 1930) and genotypic sex determination is generally believed to occur in the other Laminariales (Smith, 1955).

Naylor (1956) has given the most recent and most comprehensive description of the gametophytic nuclear division in the Laminariales, based on her studies of Laminaria digitata, Laminaria ochroleuca, and Laminaria saccharina. Her descriptions do not differ basically from those given for Egregia menziesii (Myers, 1928) and Pterygophora californica (McKay, 1933). Naylor stated that the resting nucleus is granular with one or two larger, more deeply staining granules and a conspicuous nucleolus. With the onset of prophase, the nucleus enlarges and the chromosomes take on a fine threadlike appearance. These threadlike chromosomes gradually condense to form rods and then spheres and the nucleolus disappears. By prometaphase the nuclear membrane has broken down and the chromosomes are fairly widely separated. The metaphase chromosomes appear circular in polar view and elongated in lateral view. Naylor also reported indications of a spindle in some instances. The chromosomes are dumbbell-shaped in the early stages of anaphase, and as this stage progresses the isthmus elongates further and finally breaks. Uniform separation of the chromosomes from the equatorial region usually follows, although there are one or two bridges resulting in laggards. In his reports on Macrocystis integrifolia (1952) and Laminaria digitata (1954) Walker indicated that the chromosomes divided while dispersed in the

cytoplasm. Naylor found no indication of this in her studies of Laminaria digitata.

The male gametophyte of the Laminariales is a fine, profusely branched structure which bears the antheridia in clusters. These clusters are reported to be restricted to branch tips in Nereocystis luetkeana (Hartge, 1928) and Pterygophora californica (McKay, 1933), while in genera studied by various authors they are located in both terminal and intercalary positions. Each antheridium liberates a single, biflagellate sperm which possesses one or two pale chromatophores and a reddish eyespot. Kanda (1939) reported the absence of an eyespot in the sperms of many of the Laminariales which he investigated. Ueda (1929) indicated that chromatophores were absent in the sperms of Laminaria religiosa. He did not mention any means of locomotion for the sperms. Liberation of the sperms has been observed in Laminaria and Chorda by Williams (1921) and Pterygophora californica by McKay (1933). Both authors reported the emergence of a single sperm from each antheridium. In Costaria costata, Angst (1927) reported that the initial antheridium continued to function as an antheridium and to liberate sperms for some time. Since she mentions smaller hyaline tips developing within the initial antheridial cells, this is probably an example of antheridial proliferation. A similar phenomenon was observed in Macrocystis integrifolia (Cole, 1959) but antheridial proliferation did not occur. Instead, after the liberation of the first sperm, a second sperm produced by the intercalary cell supporting the antheridium passed into the empty antheridium and out through the same aperture as the first.

The female gametophyte of the Laminariales consists of fewer and stouter cells and exhibits less branching than the male gametophyte.

Terminal cells and one-celled lateral branches are transformed into oogonia. At maturity a single egg is extruded from each oogonium and usually remains attached to the tip of the oogonial wall until after fertilization has taken place and the young sporophyte has begun to develop. Fertilization was reported to occur before extrusion of the egg in Macrocystis pyrifera (Levyns, 1933), Macrocystis integrifolia (Walker, 1952) and Laminaria digitata (Walker, 1954). Angst (1929) also noted the same phenomenon once in Pleurophycus gardneri.

Although the morphological details of fertilization were reported for Laminaria and Chorda (Williams, 1921), the cytological details were not observed until 1933 in Pterygophora californica (McKay), and 1939 in Eisenia arborea (Hollenberg). In all cases a single sperm effected fertilization. Immediately upon cytoplasmic union of egg and sperm a distinct wall formed about the zygote and a bulge was evident at the point of sperm entry. Wall formation has been noted in numerous other investigations where fertilization was not observed. Hollenberg (1939) was able to see the chromosome complement of both the egg and the sperm just prior to nuclear fusion. He noted that the zygote nucleus reverted to interphase before it underwent its first mitotic division. McKay (1933) reported a slightly different sequence in Pterygophora californica. The sperm nucleus was in early prophase as it entered the egg and the egg nucleus advanced to this stage before karyogamy. The union of the egg and sperm nuclei was accomplished because the intervening nuclear membranes dissolved. A nuclear membrane formed about the fused nuclei before a regular mitotic division occurred. Walker (1954), however, reported a type of endomitosis in Laminaria digitata which resulted in a very complex polyploid condition. Consequently he believed a sorting out of the respective

chromosome sets occurred when wall formation began at the four-, eight-, or sixteen-nucleate stage. Naylor (1956) considered this phenomenon to be an abnormal condition. Previous results concerning the development of the young sporophytes of Laminaria digitata (Kylin, 1918; Schreiber, 1930) support Naylor's claim of regular wall development following each nuclear division.

Following the pattern of development reported by the majority of workers, divisions continue in one plane to produce a uniseriate filament of from four to twelve cells. Divisions then begin in a second plane finally resulting in a flat, monostromatic thallus of several hundred cells. Rhizoids form as elongations of the basal cells and are colorless and non-septate. Divisions of the cells in a third plane give thickness to the young sporophyte. In his interfertility studies with Laminaria digitata Sundene (1958) has cultured sporophytes up to 5 - 6 cm in length. There are, however, no reports of complete growth to a reproductive state in culture.

Mitosis in the sporophyte is essentially the same as that reported for the gametophyte (Naylor, 1956). Duplication of the chromosomes while dispersed in the cytoplasm has been reported for the sporophyte (Walker, 1954). Naylor was unable to find evidence of this in her studies. The mitotic chromosome counts reported by both Walker and Naylor are included in Table 1.

Parthenogenetic development of unfertilized eggs has been described by Schreiber (1930) for Laminaria digitata and Laminaria hyperborea. The haploid "sporophytes" did not follow the regular pattern of development, but divided in an irregular manner and gave rise to aberrant forms. Myers (1928) reported that parthenogenesis did not occur in Egregia menziesii,

since extruded eggs did not develop in cultures where male gametophytes failed to liberate sperms.

11 Nereocystis luetkeana (Mertens) Postels and Ruprecht

Nereocystis is a monotypic genus of the family Lessoniaceae in the order Laminariales. This alga, commonly known as the "bull kelp", is confined to the west coast of North America. It occupies exposed as well as sheltered areas of the subtidal zone, from Santa Barbara, California to Shumagin Island, Alaska (Scagel, 1957), and grows extensively in the Puget Sound region. Some early investigators (Frye, 1916; Hartge, 1928) considered Nereocystis to be a perennial with the conspicuous phase reaching the surface of the water in the second year of development. This plant is now believed to be an annual with the stipe and lamina together reaching lengths up to 30 meters in one growing season. (Setchell and Gardner, 1925; Scagel, 1948; Smith, 1955). Nereocystis attaches either to rocky substrates or to other large kelps such as Pterygophora (Setchell, 1908) by means of a holdfast consisting of repeatedly dichotomized haptera. Extending from the holdfast is an elongated stipe which reaches to the surface of the water at maturity, where it terminates in an ovoid pneumatocyst. The pneumatocyst and terminal portion of the stipe are hollow and are filled primarily with carbon monoxide (Frye, 1916; Blinks, 1951). The single frond of the immature plant develops by means of a transition zone and subsequently splits dichotomously into several lamina by a series of preformed splits extending into the transition zone. In mature plants these lamina bear sori on both surfaces. Each sorus is an aggregation of sporangia and paraphyses, or sterile filaments.

The life-cycle of Nereocystis agrees with the life-cycles reported for other members of the Laminariales (Fig. 1), and since Hartge's (1928)

demonstration of morphological alternation of generations she and subsequent workers have considered the conspicuous phase to be the diploid, sporophytic generation. This had not been established cytologically, however, up to the time of the present investigation.

B. MATERIALS AND METHODS

Mature sori of Nereocystis luetkeana were collected during the summers of 1957, 1958, and 1959 in Vancouver Harbour, British Columbia, and in the vicinity of the Friday Harbor Laboratories of the University of Washington at Friday Harbor, Washington. These were used to start cultures of gametophytes according to the method of Hollenberg (1939). The fertile pieces were allowed to dry for periods of 2 - 3 hours, wiped free of gross surface contamination and then quickly but thoroughly rinsed in sterile sea water. They were next placed in Erdschreiber solution (Starr, 1956) for a brief period, during which time imbibition (after the partial drying) aided in the liberation of zoospores. A drop of the solution was then observed microscopically, since the presence of motile spores indicated that the sorus used was fully mature. Approximately 20 cc of the nutrient solution containing actively motile spores were aseptically pipetted into a slide tray containing 20 slides and 100 cc of nutrient solution. The culture solution was changed every 4 - 6 days.

Temperature is an important factor in the production of fertile gametophytes in culture, since gametophytes grow vegetatively at 14 - 20° C., but fail to become sexually mature. Cultures started in June, 1959 at Friday Harbor, were transferred within 48 hours to the cold chamber at the University of British Columbia. The temperatures in the chamber ranged from 6 - 9° C., and fluorescent lighting was controlled to give 12 hours of light and 12 hours of darkness. The light intensity at the level of the cultures was between 40 and 60 foot-candles. Within 33 days after initiation of the cultures the gametophytes were producing eggs and sperms.

Fertile material from these cultures was fixed at various times of the artificial day in 3 : 1 (ethanol: acetic acid) solution, to which had

been added a few drops of IKI solution. The IKI aided in fixation and also served as a decolorizing agent for the chromatophores. The fixed material was stored for at least 24 hours before being stained. The slides were removed from the fixative, rinsed in tap water, and placed in a 2% iron-alum solution for 15 minutes at room temperature. They were then inclined at about a 45° angle and flooded with acetocarmine until the red-grey color disappeared, indicating that the excess alum was removed. Slides rinsed in tap water after the iron-alum treatment and before the addition of the stain did not stain as intensely or as specifically. Cover slips were applied and the slides were warmed over a spirit lamp before sealing with a paraffin-gum mastic compound.

Sori of Nereocystis collected in various stages of development during 1957 through 1959 were fixed in 3 : 1 solution at the time of collection and later transferred to 70% ethanol. Preliminary slides of free-hand or freezing-microtome sections were made to find material in suitable stages of development. Such material was cut into small pieces, dehydrated by the tertiary-butyl alcohol method (Johansen, 1950), and embedded in paraffin (MP 56 - 58° C.). Sections were cut at a thickness of five microns using a rotary microtome. These were subsequently stained by a modified acetocarmine technique. The hydrated slides were mordanted for 15 minutes at room temperature in 2% alum. They were then placed in preheated acetocarmine (60° C.) for 15 - 20 minutes. The stained slides were run through an ethanol-xylene series and cover slips applied using gum damar mountant.

Photomicrographs were taken using 35 mm Kodak High Contrast Copy (Kodak Microfile) and Kodak D-11 developer, for maximum contrast. Prints

were made on Kodabromide F3, single weight paper and developed in Cobrol. Free-hand drawings were made directly from the microscope in order to facilitate counting the chromosomes of Nereocystis luetkeana.

C. OBSERVATIONS

I Meiosis and the Production of Zoospores

Nereocystis luetkeana was found to follow the pattern of zoospore development previously determined for various other members of the Laminariales. The initial division of the zoosporangial nucleus is reductional. The premeiotic nucleus of a young sporangium is granular, possesses one nucleolus, and measures ca. 3 microns in diameter (Fig. 2). As prophase of the first division of the sporangial nucleus commences, the nucleus enlarges to 6 - 7 microns in diameter and the chromatic material, now visible as a reticulum, concentrates at one side of the nucleus (Fig. 3): this configuration is similar to synizesis of higher plants. The nucleolus is located in the clear central area (Fig. 3). Chromatic threads are seen connecting the nucleolus with the synizetic knot. Following synizesis the chromatic threads disperse throughout the nucleus and appear as a series of beaded threads. It is not known whether it is justifiable to call the dispersed chromatic threads a "spireme", since the continuous nature of the reticulum could not be observed. Synapsis was not observed as the nuclei are small and the chromatic threads are near the limit of microscopic resolution. With condensation of the chromatic threads distinct chromosomes are seen which have configurations characteristic of diplotene. One O-shaped and four or five V-shaped elements are visible at this stage (Fig. 4 & 5). After further condensation these shapes are no longer evident and a fairly wide separation of ca. 31 bivalents occurs at diakinesis. A compact aggregation of the chromosomes results in the formation of the metaphase configuration (Fig. 6). A few cells show indications of a spindle apparatus. A centrosome-like body was observed at one pole

in a single instance (Fig. 6). Bridges are frequently seen in the early stages of anaphase as the metaphase clump divides into two smaller aggregations (Fig. 7). The two anaphase clumps proceed towards opposite ends of the sporangium. The first indication of telophase is a crescent-shaped formation of the chromatic material (Fig. 8) which later becomes circular. With the reversion to interphase the chromatin loses its stainability, the nuclear membrane reforms, and the nucleolus reappears. The paucity of cells in this stage indicates that it is not of long duration, and the second division follows quickly after the first.

The chromatic threads of prophase II condense to form a series of similarly shaped chromosomes. Although accurate counts are impossible, there appear to be ca. 26 - 31 chromosomes at this stage (Fig. 9). Clumping of the chromosomes, as seen in Figure 10, produces metaphase II. Anaphase II follows as the simultaneous separation of the metaphase aggregates. A single centrosome-like body was seen in one anaphase configuration (Fig. 11). Chromatic-crescents are evident again at early telophase II (Fig. 12), and are followed by reversion to interphase to produce the four-nucleate stage (Fig. 13). Numerous four-nucleate sporangia were seen, indicating an interphase of some duration.

The third nuclear division within the zoosporangium is mitotic and synchronous, and results in eight nuclei (Fig. 14). Two further mitotic divisions, which are often simultaneous (Fig. 16 & 17), give rise to 32 nuclei in a mature zoosporangium (Fig. 18 & 19). At this time the zoosporangium is extensively vacuolated and fusion of these vacuoles cleaves the cytoplasm into 32 uninucleate protoplasts. Metamorphosis of the protoplasts into zoospores then occurs and each zoospore possesses 2 flagella, a nucleus, a chromatophore, and some cytoplasm.

The zoospores are liberated following the rupture of the gelatinous tip of the sporangium (Fig. 20). The zoospores emerge in a group as a "spurt", a process also described by Schreiber (1930). They remain as a group at the tip of the sporangium for a few seconds, then each zoospore actively but erratically swims away using its two unequal flagella (Fig. 21 & 22). The longer flagellum is directed forward and the shorter one backward. The zoospore is pyriform with a single large chromatophore in the larger posterior end and a centrally located eyespot adjacent to the chromatophore. The nucleus is located in the smaller anterior end.

In culture, the zoospore remains active for periods varying from a few minutes to a few hours, before settling down. The longer anterior flagellum attaches first and the zoospore can be seen to pull violently against this attachment. Five minutes of this type of activity is usually noted before movement ceases. The spore then rounds up, secretes a rigid wall, and becomes quite firmly attached to the substrate. The flagella are believed to be absorbed by the spore as it attaches to the substrate.

II Early Development of the Gametophytes

The round attached spore of 4.5 - 5.5 microns develops into a gametophyte by first producing a germ tube (Fig. 23). The spore nucleus, cytoplasm, and the chromatophore usually migrate into the tube and are cut off from the optically empty spore-case by a cross-wall, thus forming the tube-cell (Fig. 24). In some instances mitosis precedes wall formation; in these instances one nucleus, most of the cytoplasm, and the chromatophore migrate into the tube, while the other nucleus and some cytoplasm remain in the spore-case and degenerate. The spore-case and its disintegrated contents take no further part in gametophytic development.

At higher temperatures (14 - 20° C.) the gametophytes grow vegetatively in cultures maintained for periods up to four months without becoming fertile. The fine, profusely branched filaments are considered to be male and the thicker, less branched filaments to be female. At lower temperatures (6 - 9° C.) the gametophytes show an entirely different mode of development. The tube-cell of the male plants has a short "resting-period" of 2 - 3 days before beginning to divide. Repeated divisions give rise to a filament consisting of five or six cells, sometimes having side branches. In some instances the tube-cell gives rise directly to 2 or 3 cells, each of which continues to produce cells, but never to the extent of the "rosette" development reported by Hartge (1928). In female plants the tube-cell rapidly enlarges from 5 microns to 15 microns in diameter, and remains as a single, large spherical cell for two or three weeks. After this "resting-period" the single cell often divides only once before becoming sexually mature, but occasionally three or four divisions occur resulting in a filament of 4 - 5 stout cells (Fig. 37). Each cell is ca. 10 - 15 microns wide and 10 microns high. The 4- or 5-celled female gametophytes do not possess as many cells as the female gametophytes obtained in cultures by Hartge (1928), possibly because of the difference in temperature conditions.

III Gametophytic Mitosis

Mitosis in the gametophytes of Nereocystis is fairly similar to mitosis as seen in higher plants. The small cell size, however, makes accurate delineation of the stages and precise chromosome counts impossible. The interphase nucleus (Fig. 34) is granular and possesses a single nucleolus. In some vegetative filaments, the interphase nucleus of an

elongated terminal or intercalary cell becomes longer (Fig. 25), and in some instances a slight constriction of the nucleus is evident. The typical nucleus undergoes a slight enlargement as prophase begins, and fine threads can be seen connecting the chromatic granules. As prophase progresses the numerous granules condense into ca. 29 - 31 short, rod-shaped chromosomes (Fig. 26 & 27; 35 & 36). These aggregate at the center of the cell to form a metaphase stage (Fig. 28). At anaphase two groups of closely associated chromosomes separate (Fig. 29). The crescent shapes of the chromatic clumps of early telophase mentioned earlier in the description of meiosis, are also evident in gametophytic mitosis. These become spherical and revert to the interphase condition prior to cytokinesis (Fig. 30). Mitotic divisions are not restricted to terminal or to intercalary cells but occur at random throughout the filament.

IV Development of Sexual Structures

i. Antheridial Development

The first indication of sexual maturity in the male gametophyte is the appearance of numerous bulges on the terminal and intercalary cells of the filament. The nucleus of the cell from which a bulge arises undergoes mitotic division and one of the daughter nuclei migrates into the bulge (Fig. 31). Cytokinesis then occurs and a one-celled branch or antheridium is produced. The nucleus of the supporting cell of the initial antheridium undergoes as many as four further divisions and the newly formed nuclei migrate into additional bulges, which are subsequently cut off by cross-walls. This results in a cluster of antheridia. Each antheridium is conical in shape with the base of the cone proximal to the filament.

Just prior to liberation of a single sperm, a clear area develops at the tip of the antheridium. The author was able to observe liberation of a sperm only once. The clear tip ruptures and a motile sperm emerges rapidly leaving the antheridium empty (Fig. 32). The sperm remains closely associated with the empty antheridium for some time and two flagella can be seen moving spasmodically. These flagella are of different lengths, although the size difference of the appendages is not as sharply pronounced as in the zoospore flagella (Fig. 33). The sperm is pyriform in shape and measures ca. 3 x 4 microns. A single pale chromatophore is located in the larger end but no eyespot is evident (Fig. 33).

ii. Oogonial Development

In sexually mature female gametophytes the terminal cells of the stout-celled filaments elongate to become oogonia. The mature and nearly mature oogonia of Nereocystis are "pear-shaped" with the expanded end proximal to the supporting cell (Fig. 38 & 39). At maturity the oogonial cytoplasm, containing several chromatophores and a single nucleus, concentrates in the narrow end of the oogonium leaving an optically clear area in the base (Fig. 38). The vegetative cells of a female gametophyte continue to divide after sexual maturity. Each new cell forms the supporting cell from which a new oogonium develops. Thus, if conditions are right and overcrowding of the cultures does not occur, gametophytes of both sexes continue to grow vegetatively and to produce reproductive structures.

Various stages of the process of liberation of the egg have been observed in living cultures as well as in fixed, stained preparations. The migration of the protoplasm to the tip of the oogonium marks the final

stage of maturation of the egg prior to its liberation. When the tip of the oogonium ruptures, approximately one-third of the egg emerges immediately (Fig. 39). Movement of the egg after this is almost imperceptible, but emergence is complete in 15 minutes. An uneven, almost spherical outline is exhibited by the egg at this time in both living and stained preparations. Stained preparations reveal the presence of a granular nucleus and a single nucleolus (Fig. 40).

iii. Fertilization and Development of the Young Sporophyte

Sperms in the immediate vicinity are attracted to the extruded egg. It is probable that a chemical substance is given off by the developing oogonium, since sperms can be seen clustered around immature oogonia, as well as around the unfertilized eggs (Fig. 41). Lateral attachment by the sperm is followed by the formation of a cytoplasmic bridge (Fig. 42) and the sperm enters the egg. The fate of the flagella is unknown, but they are present up to the time of cytoplasmic union. A wall develops around the dikaryotic cell immediately after fusion, and a bulge at the point of sperm entry becomes very noticeable (Fig. 43). The cytoplasm of the sperm cannot be distinguished once the sperm is inside the egg. The sperm nucleus is in interphase at the time of entrance as is the egg nucleus (Fig. 44) and they both remain in this condition until karyogamy is complete. During fertilization the egg loses its spherical shape and gradually becomes elliptical with the long axis directed in the plane of the empty oogonium. Karyogamy is accomplished by the migration of the sperm nucleus to the egg nucleus and their subsequent fusion (Fig. 45). As the egg and sperm nuclei gradually coalesce there is complete dissolution of the nuclear membranes. Fertilization is completed when the nucleoli

fuse and a new nuclear membrane forms. The zygote nucleus is in the granular interphase and contains a single nucleolus (Fig. 46).

The zygote nucleus begins to divide in a manner similar to gametophytic mitosis. The granular threads of early prophase contract to produce ca. 58 - 62 chromosomes at prophase. These counts are double those observed in the gametophytes (Fig. 47 & 48; 55 & 56). The chromosomes at this time appear dumbbell-shaped in lateral view and it is assumed that they divide at the isthmus in the early stages of anaphase. After the chromosomes move to the poles at anaphase, the crescent-shaped chromatic lumps of early telophase are apparent (Fig. 49). These chromatic clumps become spherical and revert to the interphase condition, with each daughter nucleus possessing a single nucleolus (Fig. 50). Cytokinesis then occurs. The nucleus of the terminal cell usually divides next, but cross-wall formation does not always follow karyokinesis immediately. Cytokinesis sometimes awaits the mitotic division of the basal cell after which both walls form simultaneously (Fig. 51 - 54). Further transverse divisions are random with any cell participating, until a 5 - 8 celled uniseriate filament is attained. A bi-nucleolate condition was observed in cells just prior to nuclear division, indicating that nucleolar duplication occurs before actual nuclear division takes place. Central cells of the filament then divide vertically followed by vertical divisions of all but the basal cell. Divisions in the two planes continue, producing a flattened, monostromatic thallus. Rhizoids appear at the same time as divisions in a third plane give rise to a di- and then a tristromatic sporophyte. The rhizoids develop as elongations of the basal cells, and are non-septate and colorless. Cultures at six months

had sporophytes of a thickness of three cells and a length of 8 mm.

Cytokinesis in the early sporophyte begins with the formation of a clear area in the center of the cell between the interphase nuclei. This area is bisected by a series of granules (Fig. 52) which fuse to form a definite division between the nuclei. The initial band between the nuclei is the middle lamella. Primary thickenings are first seen in the middle regions and these proceed centrifugally to the lateral walls.

Some unicellular "sporophytes" were seen which had wall development completed, yet had only the haploid number of chromosomes. In comparison to the normal diploid sporophytes, the prophase stage of the haploid "sporophytes" (Fig. 60 - 61) is considered to be relatively extended on the basis of the number of prophase stages of each type observed. Nuclear division of the haploid "sporophytes" proceeds in an aberrant fashion and cytokinesis is not synchronized to any degree with karyokinesis. These irregularities result in deformed plants, limited in growth to a few multinucleate cells (Fig. 62 - 65). The stunted, deformed growth of these parthenogenetic "sporophytes" indicates that development to mature spore-producing Nereocystis plants is unlikely.

D DISCUSSION

I Spore Formation and Liberation

It has been pointed out that 32 nuclei develop in the sporangium of Nereocystis as a result of one meiotic sequence and three mitotic divisions. When these divisions are complete, each nucleus, in association with some cytoplasm and a chromatophore, forms a zoospore. This is accomplished by fusion of vacuoles, a process previously reported for Pterygophora californica (McKay, 1933) and for Eisenia arborea (Hollenberg, 1939).

Prior to liberation of the zoospores there is an enlargement and elongation of the sporangium which forces its way between the paraphyses. Rupture of the tip of the sporangium creates an aperture through which the zoospores are liberated as a group. Zoospores are not liberated from isolated sporangia, an observation also reported by Schreiber (1930). Thus, like him, I am led to the conclusion that the osmotic pressure changes within a single sporangium do not in themselves cause the liberation of the zoospores. Pressures developed by the contiguous cells must also play a major role.

The surfaces of the sori of Nereocystis are covered by a gelatinous sheath which "traps" the zoospores as they emerge from the sporangia. The sheath then rapidly dissolves and the zoospores are able to swim away freely. The gelatinous sheath is largely removed by wiping the sorus prior to the initiation of cultures, since it is a source of much contamination, mainly by diatoms and bacteria.

The motile zoospores remain active for a brief period and then attach to the substrate. Non-motile spores of Nereocystis (possibly immature or over-mature zoospores) did not attach to the perpendicular surface of the

glass slides in the culture dishes. Since the flagella are the first organs to attach, a thigmotactic response is believed to be involved. Once attached, the spore rounds up and develops a wall. Following production of a germ tube, gametophytic development proceeds according to the pattern previously described.

II Temperature Conditions and Sexual Maturity

While this study has been primarily concerned with nuclear divisions and fertilization, observations have been made over the past three years regarding temperature requirements for the successful production of sexual stages in the gametophytes of Nereocystis. The temperature requirements for vegetative growth and for reproductive development do not coincide in Nereocystis, but rather follow in general the findings of Schreiber (1930) for Laminaria digitata, Laminaria saccharina, and Laminaria hyperborea. Temperatures of 14° C. allowed vegetative growth to proceed but "inhibited" the development of sexual organs. Transfer of 6-week-old cultures to temperatures of 6 - 9° C. promoted sexual development within one week. Controls kept at ca. 14° C. continued to grow vegetatively but did not become fertile. These observations do not appear readily reconcilable to the report of Hartge (1928) who obtained fertile Nereocystis gametophytes at a mean temperature of 16° C. Cole (personal communication) was able to grow Nereocystis gametophytes to sexual maturity in 1957 using cultural conditions similar to those used by Hartge. She feels, however, that the temperatures were generally cooler than those observed by Hartge. Since Hartge's cultures did not become sexually mature until September, it seems likely that temperatures did drop to the necessary critical level at that

time. It is suggested that the upper critical level at which sexual structures are produced in Nereocystis gametophytes is 10° C.

The whole question of induction of sexuality in the Laminariales raises problems that go beyond the scope of present knowledge. Is temperature the primary factor in sexual maturation of Nereocystis gametophytes or does it merely act secondarily by superimposing its effects over more basic factors? The uptake of materials required to convert sterile gametophytes into sexually mature plants may be regulated by temperature levels. On the other hand, temperature conditions may exert a control over the metabolic pathways, and cooler temperatures may be necessary to open up or block pathways causing the production or accumulation of substances required for sexuality. It would be interesting to grow parallel cultures at various temperatures for two reasons; first, to determine a critical temperature for sexuality and second, to utilize reproducing individuals for the purpose of determining whether a diffusible substance is produced which will induce sexuality in sterile gametophytes, even under "adverse" temperature conditions. The difficulty of obtaining the unialgal cultures that would be required for such a study can be overcome with relative ease by using zoospores to initiate the cultures. Bacteria-free cultures would also be essential, particularly if the work were to proceed to the stage of chemical isolation and identification of the "stimulating" substance.

III Determination of Sex in Gametophytes

Although actual experimental evidence is lacking in Nereocystis, genotypic sex determination appears to occur in the sporangium at meiosis. In cultures set up from a single sorus, the zoospores develop into approximately equal numbers of male and female gametophytes. Such an observation is not proof

of genotypic sex determination, but if the numerical equality of the sexes from a single sorus requires explanation, the alternate possibilities do not appear plausible. One such alternate is that each sporangium develops exclusively male or exclusively female gametophytes. This would require some mitotic segregation of sex determining factors or genes. Since this is not known as a regular occurrence in detailed studies of other organisms, it seems unlikely that such is the case. Another alternative is the influence of the environment, but since male and female gametophytes are readily distinguishable at an early age under the same environmental conditions, the influence of the environment can be discounted as a factor involved in the actual determination of sex. Further supporting evidence is found in the fact that genotypic sex determination exists in Laminaria saccharina (Schreiber, 1930).

IV Division Sequence and Chromosome Number

The technique of culturing the gametophytes of the Laminariales has been used to increase the basic knowledge regarding alternation of generations both from a morphological and a cytological point of view. The present investigation has added Nereocystis luetkeana to the increasing numbers of this group for which the cytological basis has been shown for the morphological alternation of generations. A haploid chromosome number of ca. 31 has been found. This count corresponds closely with Naylor's (1956) counts for Laminaria digitata, Laminaria ochroleuca, and Laminaria saccharina, each with 27 - 31 as the haploid number, and also Nishibayashi and Inoh's (1957) count of N equals 30 for Costaria costata. Although the methods for handling small chromosomes are not completely adequate, the counts among the Laminariales (Table I) appear to align themselves into three main groups, differing by approximately 10. These results could reflect a polyploid series. The

inability to draw definite conclusions regarding chromosome levels and interrelationships within the Order, or to show any phylogenetic trends is, of course, a direct result of the lack of evidence. The present investigation has indicated that, with new and repetitive cytological studies, coupled with intra- and interspecific fertility studies, evolutionary and taxonomic relationships will undoubtedly become more apparent.

During the investigation of the division sequences some apparent abnormalities were seen. Centrosome-like bodies were observed in two instances during meiosis; once at a single pole of metaphase I and again at a single pole of anaphase II. Centrosomes have been reported for various species of the Laminariales, but not for all those studies cytologically (see above, p.2). In Nereocystis, these "centrosomes" were seen in neither sporophytic nor gametophytic mitotic divisions. Rather than centrosomes, the objects seen in this investigation could well have been precocious chromosomes or lagging chromosomes which have failed to orient at the metaphase region. The infrequent occurrence of these structures does not make it possible to account for them on the basis of sex chromosomes. Rather they are regarded as isolated division abnormalities.

In normal and abnormal (parthenogenetic) sporophytes the nuclei often possessed two nucleoli. This is not an abnormal condition but rather a regular feature of sporophytic karyokinesis. Prior to the onset of prophase the nucleoli divide or are duplicated in some manner, although there is no evidence of chromosome replication at this time. It can be imagined that such chromosome replication is taking place and is manifested in the appearance of two nucleoli. Another interpretation is that the nucleolus is manufacturing or storing RNA (ribonucleic acid). This is then evidenced by

the division of the nucleolus, or possibly as the formation of a second nucleolus, prior to chromosome replication. This could mean that RNA plays a vital part in the replication process. The close correlation between the appearance of two nucleoli and nuclear division can only be followed accurately for the first three divisions of the sporophyte. In all cases the nucleus which was about to divide exhibited two nucleoli, while the other nucleus had only one. Interphase nuclei seen at later divisions, where wall formation had not yet occurred, possessed but a single nucleolus.

V. Parthenogenetic Development

Two possibilities are suggested for the occurrence of parthenogenetic development of eggs in cultures where fertile male gametophytes are in abundance. The first and most obvious is the idea that this is a cultural artifact, a phenomenon brought on by the conditions of the culture technique. Parthenogenetic development has been observed in the Laminariales at least once before (Schreiber, 1930) in culture. This could mean that parthenogenetic development occurs freely or occurs only in culture; further investigations are required to determine which is the case. Another possibility is that there is a critical time in which fertilization can occur. The time factors involved are not known, but an unfertilized egg has four possible pathways open to it: (1) it can degenerate; (2) it can secrete a wall, begin to develop parthenogenetically and give rise to a sporophytic plant; (3) it can give rise to a gametophytic filament (no evidence of this was seen); (4) it can be fertilized and follow the typical pathway of sporophytic development. Continued development of unfertilized eggs as sporophytes does not occur (Fig. 60 - 65). From observations of the number

of countable prophases encountered among the one-celled sporophytes it is apparent that parthenogenetic development slows down the prophase of the initial division. In subsequent divisions karyokinesis is not in phase with cytokinesis. The resulting plantules are very stunted, and exhibit 2 or 3 multinucleate cells at the age of five months. This compares to two or three hundred cells in the typical sporophytes. There is apparently no way of overcoming the parthenogenetic mode of development once it is initiated. Although fusion of the haploid nuclei in the multinucleate cells could conceivably lead to direct diploidization and "normal" development thereafter, no evidence of this was seen in five-month-old cultures.

SUMMARY

Sori of Nereocystis luetkeana in various stages of development were collected and fixed in 3:1 solution. These were embedded, sectioned, stained with acetocarmine and subsequently observed for the karyokinetic sequences involved in the formation of zoospores. The first division sequence of the zoosporangial nucleus is meiotic and shows a bivalent count of 31. Three more or less synchronous mitotic divisions follow, resulting in 32 nuclei in a nearly mature sporangium. Thirty-two zoospores are seen to emerge from a mature sporangium.

Mature sori of Nereocystis were used to establish cultures of gametophytes. The gametophytes follow in general the developmental sequence reported for other Laminariales; the spore produces a germ tube and the nucleus usually migrates into the tube prior to nuclear division. The tube-cell is the basis for further development since the spore-cell gradually degenerates. Mitosis in the gametophytes is typical of that seen in higher plants and occurs in terminal and intercalary cells of the filaments. Temperature is an important factor in the production of sexually mature gametophytes. Cooler temperatures (less than 10° C.) are necessary to initiate sexual development, while higher temperatures "inhibit" sexuality but allow vegetative growth to proceed.

Antheridia are produced as clusters of one-celled branches of both terminal and intercalary cells of the fine, many-celled male gametophytes. Each antheridium produces a single sperm. The terminal cells of the larger-celled, less extensive female gametophytes develop into oogonia. Oogonia are also produced as one-celled branches of intercalary cells. A single egg is extruded from each oogonium and is fertilized by a single sperm, thus reconstituting the diploid complement of ca. 62 chromosomes.

Cells of the sporophyte divide transversely to produce a uniseriate filament of from 5 - 8 cells before divisions in a second plane produce a flat, monostromatic thallus. Divisions in a third plane begin at the time of rhizoid development. Sporophytes 8 mm long and consisting of 200 - 300 cells were obtained in 6-month-old cultures.

Some one-celled sporophytes were observed with the haploid number of 31 chromosomes. These were believed to be the initial stages of parthenogenetic development which subsequently gave rise to 2 or 3 celled, multinucleate "sporophytes". Growth of these stunted plants to mature, sporulating Nereocystis plants seem unlikely.

LITERATURE CITED

- Abe, K. 1939, Mitosen im Sporangium von Laminaria japonica Aresch. Sci. Rep. Tohoku Univ. 14: 327 - 329
- Angst, L. 1927, Gametophytes of Costaria costata. Publ. Puget Sound Biol. Sta. 5: 293 - 307
- Angst, L. 1929, Observations on the development of zoospores and gametes in Pleurophycus Gardneri. Publ. Puget Sound Biol. Sta. 7: 39 - 48
- Blinks, L. R. 1951, Physiology and Biochemistry of Algae, in Manual of Phycology, Chronica Botanica, Mass. U. S. A.
- Cole, K. 1959, Developmental studies of certain marine algae in culture. International Oceanographic Congress Preprints, 195 - 196. New York
- Frye, T. C. 1906, Nereocystis luetkeana. Bot. Gaz. 42: 143 - 146
- Frye, T. C. 1916, Gas pressure in Nereocystis. Publ. Puget Sound Biol. Sta. 1: 85 - 88
- Hartge, L. A. 1928, Nereocystis. Publ. Puget Sound Biol. Sta. 6: 207 - 237
- Herbst, C. C. & G. R. Johnstone, 1937, Life history of Pelagophycus porra. Bot. Gaz. 99: 339 - 354
- Hollenberg, G. J. 1939, Culture studies of marine algae. I Eisenia arborea. Amer. Jour. Bot. 26: 34 - 41
- Inoh, S. & T. Nishibayashi, 1954, On the mitosis in the sporangium of Undaria pinnatifida (Harv.) Sur. Biol. Jour. Okayama Univ. 1: 217 - 225
- Johansen, D. A. 1950, Plant Microtechnique, McGraw-Hill Book Co. New York
- Kanda, T. 1936, On the gametophytes of some Japanese species of Laminariales. Sci. Papers Inst. Algal Res., Fac. Sci. Hokkaido Imp. Univ. 1: 221 - 263
- Kylin, H. 1916, "Über den Generationswechsel bei Laminaria digitata. Svensk. Bot. Tidskr. 10: 551 - 561
- Kylin, H. 1918, Studien über die Entwicklungsgeschichte der Phaeophyceen. Svensk. Bot. Tidskr. 12: 1 - 64
- Levyns, M. R. 1933, Sexual reproduction in Macrocystis pyrifera Ag. Ann. Bot. 47: 349 - 353
- Mange, F. 1953, Meiosé et nombre chromosomique chez les Laminariaceae (Laminariales, Phaeophyceae). C. R. Acad. Sci. (Paris) 236: 515 - 517

- McKay, H. H. 1933, The life history of Pterygophora californica Ruprecht. Univ. Calif. Publ. Bot. 17: 111 - 147
- Myers, M. E. 1928, The life history of the brown alga Egregia menziesii. Univ. Calif. Publ. Bot. 14: 225 - 246
- Naylor, M. 1956, Cytological observations on three British species of Laminaria: a preliminary report. Ann. Bot. 20: 341 - 347
- Nishibayashi, T. & S. Inoh, 1956, Morphogenetical studies in the Laminariales I. The development of zoospores in Laminaria angustata Kjellm. Biol. Jour. Okayama Univ. 2: 147 - 158
- Nishibayashi, T. & S. Inoh, 1957, Morphogenetical studies in Laminariales II. The development of zoosporangia and the formation of zoospores in Costaria costata (Turn.) Saunders. Biol. Jour. Okayama Univ. 3: 169 - 181
- Sauvageau, C. 1915, Sur la sexualité hétérogamique d'une Laminiaire (Sacchoriza bulbosa). C. R. Acad. Sci. (Paris) 161: 796 - 798
- Sauvageau, C. 1916a, Sur les gamétophytes de deux Laminaires (L. flexicaulis et L. saccharina). C. R. Acad. Sci. (Paris) 162: 601 - 604
- Sauvageau, C. 1916b, Sur la sexualité hétérogamique d'une Laminiaire (Alaria esculenta). C. R. Acad. Sci. (Paris) 162: 840 - 842
- Scagel, R. F. 1948, An investigation on marine plants near Hardy Bay, B. C. Rep. to Prov. Dept. Fish. 1
- Scagel, R. F. 1957, An annotated list of the marine algae of British Columbia and Northern Washington. Nat. Mus. Can. Bull. 150. (Biol. Series 52)
- Schreiber, E. 1930, Untersuchungen über Parthenogenesis, Geschlechtsbestimmung, und Bastardierungsvermögen bei Laminarien. Planta 12: 331 - 353
- Setchell, W. A. 1908, Nereocystis and Pelagophycus. Bot. Gaz. 45: 125 - 134
- Setchell, W. A. & N. L. Gardner, 1925, The marine algae of the Pacific Coast of North America. III. Melanophyceae. Univ. Calif. Publ. Bot. 8: 383 - 898
- Smith, G. M. 1955, Cryptogamic Botany, Vol. 1. Algae and Fungi. McGraw-Hill Book Co., New York, U. S. A.
- Starr, R. C. 1956, Culture collection of algae at Indiana University. Lloydia 19: 129 - 149
- Sundene, O. 1958, Interfertility between forms of Laminaria digitata. Nytt. Mag. Bot. 6: 121 - 128

- Ueda, S. 1929, On the temperature in relation to development of the gametophytes of Laminaria religiosa Miyabe. Jour. Imp. Fish. Inst. Tokyo 24: 138 - 139
- Walker, F. T. 1952, Chromosome number in Macrocystis integrifolia Bory. Ann. Bot. N. S. 16: 23 - 27
- Walker, F. T. 1954, Chromosome number of Laminaria digitata Lamour. Ann. Bot. N. S. 18: 113 - 120
- Williams, J. L. 1921, The gametophytes and fertilization in Laminaria and Chorda (Preliminary account). Ann. Bot. 35: 603 - 607
- Yabu, H. 1957, Nuclear division in the sporangium of Alaria crassifolia Kjellm. Bull. Fac. Fish., Hokkaido Univ. 8: 185 - 189

TABLE 1
CHROMOSOME NUMBERS IN THE LAMINARIALES

ALGA	HAPLOID NUMBER	AUTHORITY
<u>Alaria crassifolia</u>	22 a	Yabu, 1957
<u>Chorda filum</u>	20 a	Kylin, 1918
<u>Costaria costata</u>	30 a	Nishibayashi & Inoh, 1957
<u>Egregia menziesii</u>	8 ab	Myers, 1928
<u>Eisenia arborea</u>	15 ab	Hollenberg, 1939
<u>Laminaria angustata</u>	22 a	Nishibayashi & Inoh, 1956
<u>Laminaria cloustoni</u>	11 b	Walker, unpubl. (cf. Walker, 1954)
<u>Laminaria digitata</u>	8 b	Walker, 1954
<u>Laminaria digitata</u>	27 - 31 b	Naylor, 1956
<u>Laminaria flexicaulis</u>	13 a	Mange, 1953
<u>Laminaria japonica</u>	22 a	Abe, 1939
<u>Laminaria ochroleuca</u>	27 - 31 b	Naylor, 1956
<u>Laminaria saccharina</u>	13 a	Mange, 1953
<u>Laminaria saccharina</u>	27 - 31 b	Naylor, 1956
<u>Macrocystis integrifolia</u>	16 b	Walker, 1956
<u>Pterygophora californica</u>	13 ab	McKay, 1933
<u>Undaria pinnatifida</u>	22 a	Inoh & Nishibayashi, 1954

a - counts from meiotic cells
b - counts from mitotic cells

Key to Symbols Used in Figures 1 - 65

a - anaphase
ac - apical cap
an - antheridium
b - bridge
cb - chromatic body
cc - chromatic crescent
cp - cell plate
cyb - cytoplasmic bridge
e - egg
ey - eyespot
g - granules
m - metaphase
n - nucleus
nl - nucleolus
o - oogonium
p - paraphysis
pw - primary wall
r - rhizoid
s - sperm
so - sorus
sp - spindle
sy - synizetic knot
zn - zygote nucleus

Figure 1. Life Cycle of Nereocystis luetkeana

The sporophyte (i) produces 32 zoospores by a meiotic sequence followed by three mitotic divisions. These nuclear divisions take place in the sporangia (ii) which are grouped, together with the paraphyses, into fertile patches or sori. Liberated zoospores (iii) settle on the substrate, round up and develop rigid walls. Gametophytic growth begins with the production of a germ tube (iv). Subsequent growth into male (v) and female (vi) gametophytes is observed. At maturity the male produces antheridia, each of which liberates a single sperm (vii) and the female produces oogonia, each of which liberates a single egg (viii). The zygote formed by the union of egg and sperm produces a thick wall (ix) and divides to produce a 2-celled sporophyte (x) which subsequently develops into a young, multicellular sporophyte (xi). Further growth gives rise, in one growing season, to the conspicuous sporophyte (i), thus completing the cycle.

FIG. I
LIFE CYCLE OF NEREOCYSTIS LUETKEANA

Figures 2 - 10. Meiosis in the Sporangium of Nereocystis luetkeana

Fig. 2. Interphase nucleus prior to meiosis. x3000 - Fig. 3. Early prophase showing the synizetic knot, with the nucleolus situated in the central area of the nucleus. x4400 - Fig. 4. Diplotene, the nuclear membrane and nucleolus have disappeared. x4400 - Fig. 5. Diagrammatic representation of Fig. 4 showing one O-shaped and 5 V-shaped bivalents; these plus the rods and spheres total 31 bivalents. x4400 - Fig. 6. Metaphase I, with a suggestion of a spindle apparatus and a chromatic body. x3000 - Fig. 7. Anaphase I, with chromatic bridges. x3000 - Fig. 8. Early telophase I, showing chromatic crescents formed by the aggregated chromosomes. x3000 - Fig. 9. Prophase II; the chromosomes appear as rods and spheres. x3600 - Fig. 10. Metaphase II. x3000.

FIG. 2-10
MEIOSIS IN THE SPORANGIUM
OF NEREOCYSTIS LUETKEANA

Figures 11 - 19. Meiosis and Zoospore Formation in the Sporangium of
Nereocystis luetkeana

Fig. 11. Anaphase II, with a chromatic body evident at the apical pole. x3000 - Fig. 12. Early telophase II, with crescent clumps evident. x 3000 - Fig. 13. Four-nucleate sporangium. x3000 - Fig. 14. Eight-nucleate sporangium, the interphase nuclei each possess a single nucleolus. x 3000 - Fig. 15. Diagrammatic representation of Fig. 14 showing the relative positions of the eight nuclei. x3000 - Fig. 16. Evidence of non-synchronous mitotic division in the sporangium. x3000 - Diagrammatic representation of Fig. 16, showing the apical metaphase and two other divisions in anaphase. The remaining nuclei are in interphase. x3000 - Fig. 18. Nearly mature sporangium showing 32 nuclei. x2500 - Fig. 19. Diagrammatic representation of Fig. 18, showing the positions of the 32 nuclei and the incipient apical cap of the sporangium. x2500.

Figures 20 - 40. Male and Female Gametophytic Development in
Nereocystis luetkeana

Fig. 20. Mature sporangium with spores formed and the apical cap fully developed. x2500 - Fig. 21. Zoospore showing unequal flagellation. x2000 - Fig. 22. Diagrammatic representation of Fig. 21, showing the internal structure of a zoospore. x2000 - Fig. 23. A spore that has settled, rounded up, and emitted a germ tube. x2000 - Fig. 24. Tube-cell of the gametophyte. Mitosis has occurred prior to wall formation. The nuclear material in the spore-case will degenerate. x2000 - Fig. 25. Interphase nucleus in an apical cell, showing the elongated condition. x2500 - Fig. 26. Prophase in an intercalary cell of a male filament. x3000 - Fig. 27. Diagrammatic representation of Fig. 26, showing the haploid number of 31 chromosomes. x3000 - Fig. 28. Metaphase in a terminal cell of a male gametophyte. x2500 - Fig. 29. Anaphase in a terminal cell of a male filament. x2500 - Fig. 30. Interphase nuclei in an intercalary cell of a male filament prior to wall formation. Each nucleus possesses a single nucleolus. x3000 - Fig. 31. Migration of a nucleus into an intercalary bulge or developing antheridium. x3500 - Fig. 32. An empty terminal antheridium showing the antheridial walls and the liberation opening. A new bulge is developing below the empty antheridium. x3000 - Fig. 33. Diagrammatic representation of a sperm, showing the position of the chromatophore and the flagella. x2500 - Fig. 34. Single-celled female gametophyte with an interphase nucleus, showing the prominent nucleolus. x1000 - Fig. 35. Prophase in a single-celled female gametophyte. x2500 - Fig. 36. Diagrammatic representation of Fig. 35, showing a haploid number of 28 chromosomes x2500 - Fig. 37. Four-celled female gametophytes. x1000 - Fig. 38. Developing oogonium. Migration of the cytoplasm and the nucleus into the tip of the oogonium. x2000 - Fig. 39. Early stages in egg liberation; cytoplasm emerging at the tip. x2000 - Fig. 40. Fully extruded egg possessing a granular nucleus and a prominent nucleolus. x2500.

FIG. 20-40
MALE AND FEMALE GAMETOPHYTIC
DEVELOPMENT IN NEREOCYSTIS LUETKEANA

Figures 41 - 56. Fertilization and Young Sporophytes of
Nereocystis luetkeana

Fig. 41. A sperm in the vicinity of the egg. x2500 - Fig. 42. Cytoplasmic fusion between the egg and sperm. x2500 - Fig. 43. A wall formed about the zygote and a bulge is visible at the point of sperm entry. x2500 - Fig. 44. Dikaryotic cell; both nuclei are visible in the interphase condition. x2500 - Fig. 45. Nuclear fusion is proceeding; the nucleoli have not yet fused. x2500 - Fig. 46. Nuclear fusion completed with the fusion of the nucleoli. x2500 - Fig. 47. Prophase in a one-celled sporophyte. x5000 - Fig. 48. Diagrammatic representation of Fig. 47. $2N$ equals 62. x5000 - Fig. 49. Telophase of first nuclear division of the sporophyte. x3000 - Fig. 50. Two-nucleate sporophyte, both nuclei in interphase. The primary wall is developing. x3000 - Fig. 51. Late telophase of early sporophyte mitosis. x2500 - Fig. 52. Early telophase in basal cell of a sporophyte, the crescent shape is evident. Cytokinesis is progressing, and granules can be seen in the region of the cell plate. x2500 - Fig. 53. Late telophase in both cells. A cell plate is beginning to form. x2500 - Fig. 54. Four-celled sporophyte with a primary wall developing between the uppermost cells. x2500 - Fig. 55. Prophase in an intercalary cell of a six-celled sporophyte. x5000 - Fig. 56. Diagrammatic representation of Fig. 55 with a diploid number of 62 chromosomes evident. x5000.

FIG. 41-56
FERTILIZATION AND YOUNG
SPOROPHYTES OF NEREOCYSTIS LUETKEANA

Figures 57 - 65. Normal and Parthenogenetic Sporophytes of
Nereocystis luetkeana

Fig. 57. Seven-celled sporophyte showing the beginning of vertical wall formation. x3000 - Fig. 58. A flat, monostromatic thallus; cell divisions have been in two planes only. x1500 - Fig. 59. Distromatic thallus, showing the beginnings of rhizoid formation. x500 - Fig. 60. Prophase in a parthenogenetic sporophyte. x3000 - Fig. 61. Diagrammatic representation of Fig. 60, showing the haploid chromosome number of 31. x3000 - Fig. 62. Three-nucleate parthenogenetic sporophyte. x3000 - Fig. 63. Four-nucleate parthenogenetic sporophyte (4th nucleus is under the apical nucleus). x3000 - Fig. 64. Two-celled, multinucleate, parthenogenetic sporophyte. x3000 - Fig. 65. Three-celled, multinucleate, parthenogenetic sporophyte. x3000.

FIG. 57-65
NORMAL AND PARTHENOGENETIC
SPOROPHYTES OF NEREOCYSTIS LUETKEANA

FIG. I
LIFE CYCLE OF NEREOCYSTIS LUETKEANA

FIG. 2-10
MEIOSIS IN THE SPORANGIUM
OF NEREOCYSTIS LUETKEANA

FIG 11-19
MEIOSIS AND ZOOSPORE FORMATION IN THE
SPORANGIUM OF NEREOCYSTIS LUTKEANA

FIG. 20-40

MALE AND FEMALE GAMETOPHYTIC
DEVELOPMENT IN NEREOCYSTIS LUETKEANA

FIG. 41—56

FERTILIZATION AND YOUNG

SPOROPHYTES OF NEREOCYSTIS LUETKEANA

FIG. 57—65

NORMAL AND PARTHENOGENETIC
SPOROPHYTES OF NEREOCYSTIS LUETKEANA