CYTOLOGY IN TWO SPECIES OF *PORPHYRA* FROM THE STIPES OF *NEREOCYSTIS LUETKEANA* (MERT.) POST. ET RUPR.*

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During the cruise of the training ship of the Faculty of Fisheries, Hokkaido University, for the north Pacific Ocean in the summer season of 1969, I had the opportunity, in the Bering Sea, to collect and fix the leafy thalli of the species of *Porphyra* attached to the stipes of *Nereocystis luetkeana* (Mert.) Post. et Rupr. for cytological study. The sporophytes of *Nereocystis luetkeana* with the thalli of *Porphyra* on their stipes floating on the sea surface were collected on June 12th at lat. 51°04'N and long. 176°42'W, on June 17th at lat. 55°00'N and long. 165° 33'W and on July 2nd at lat. 56°30'N and long. 162°00'W. The materials collected on June 12th and 17th were identified as *Porphyra amplissima* (Kjellm.) Setch. et Hus and those on July 2nd as *P. nereocystis* Anderson from the diagnoses given by Hus in 1902. In *Porphyra amplissima*, the number of chromosomes was already reported to be 3 in haploid and 6 in diploid on the materials collected at Nemuro in Hokkaido, Japan (Kito, Yabu & Tokida, 1967), but no observation has been given yet on the behaviour of chromosomes in mitosis. When the sporophytes of *Nereocystis luetkeana* were collected, the leafy thalli of *Porphyra* were immediately picked up from the stipes of the sporophytes and they were kept alive in large vats filled with sea water in the laboratory room in the ship. Fixation was done during the hours between 8–11 p.m. Aceto-alcohol solution (1 part of glacial acetic solution to 3 parts of 95 per cent alcohol) was employed as fixative. The aceto-iron-haematoxylin-chloral hydrate solution which was recommended by Wittmann in 1965 was used for staining.

I am grately indebted to Captain T. Fujii and the crew members of the training ship "Oshoro Maru" for their kind help in collecting the materials at sea.

Results

1. *Porphyra amplissima* (Kjellm.) Setch. et Hus

Nuclear division in vegetative cell: The discrimination of the nucleus between the resting stage and the early prophase is very difficult. In vegetative cell

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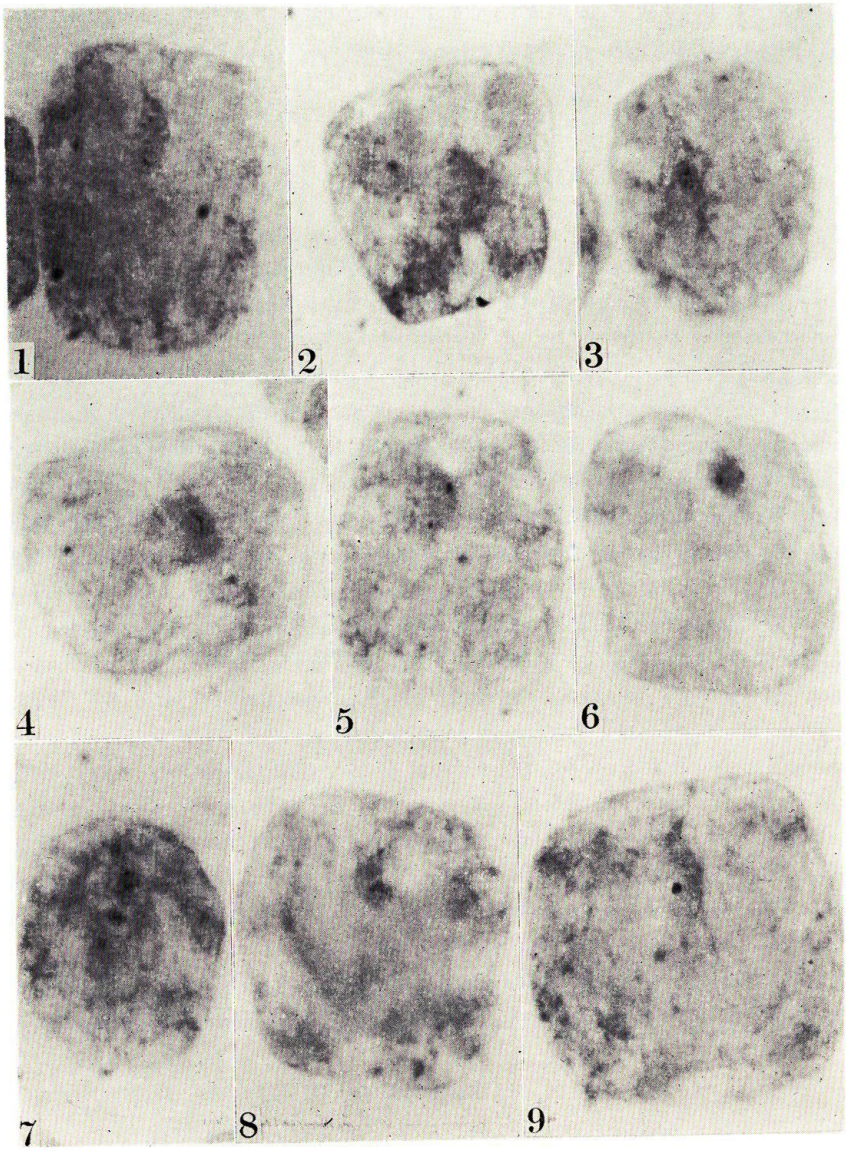
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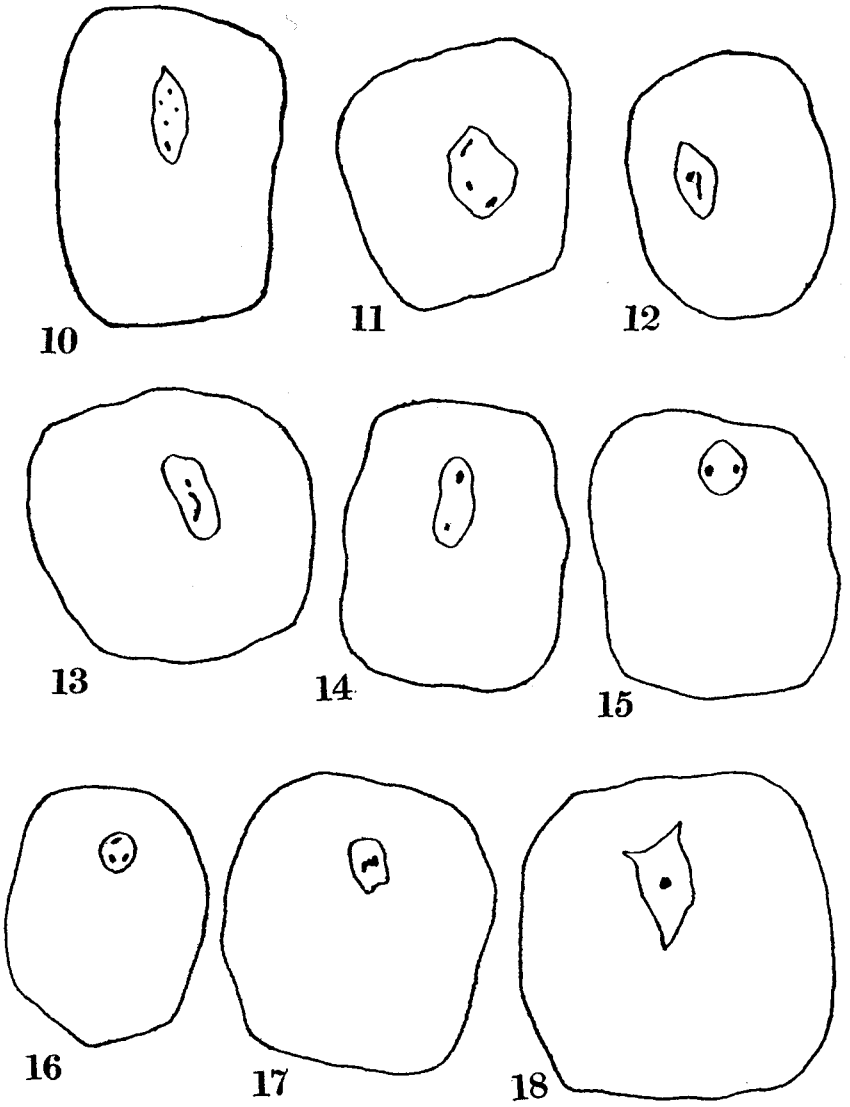
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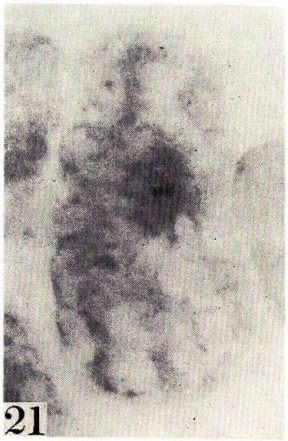
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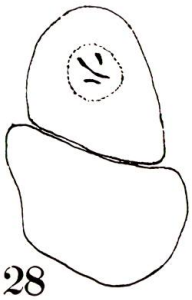
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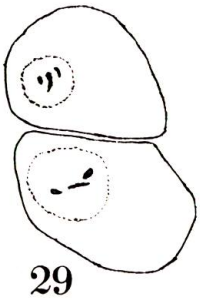
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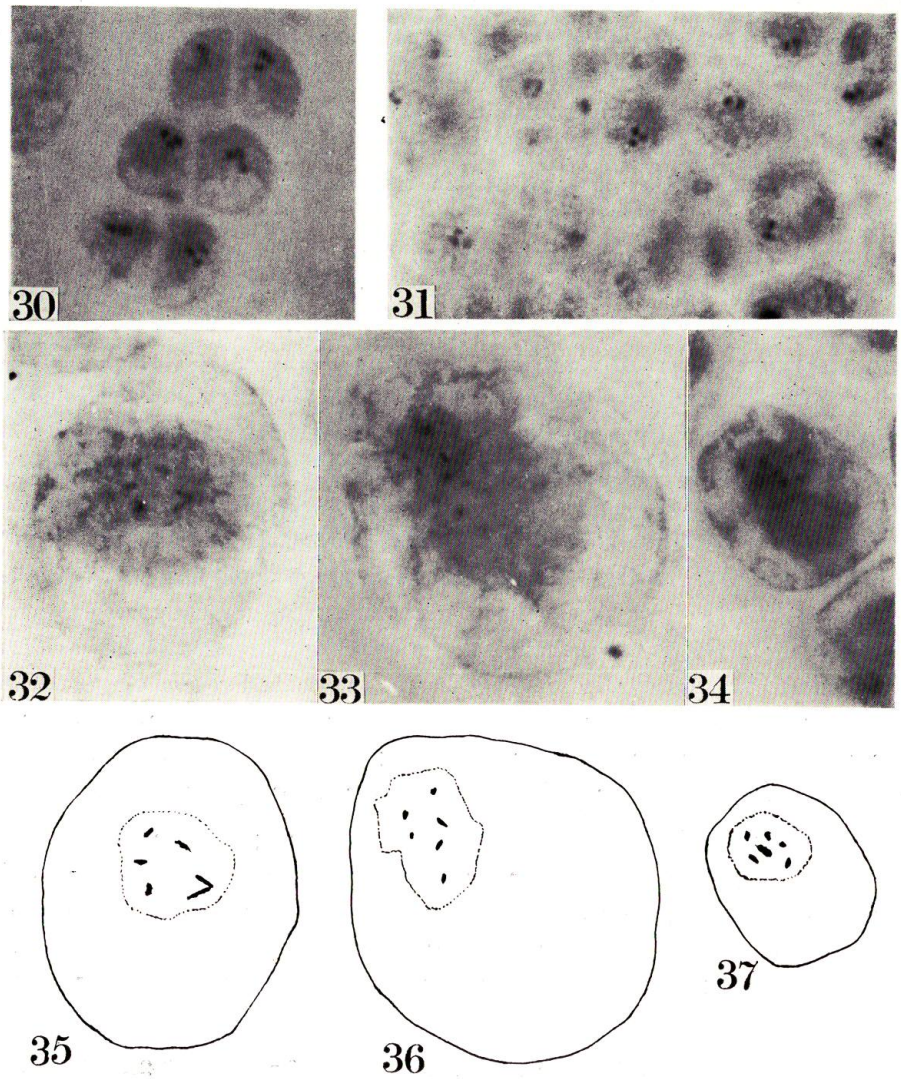

Figs. 1–37. *Porphyra amplissima* (Kjellm.) Setch. et Hus

1–9. Various stages of nuclear divisions in somatic cells; 1. Nucleus in prophase. The nucleolus has disappeared already and chromatin granules can be seen in the nuclear cavity. 2. Nucleus in prophase with three chromosomes in the nuclear cavity. 3–5. Nuclei in prophase. In each photograph two chromosomes are in the same level of focus. 7. Nucleus in early metaphase. 8. Nucleus in the side view of metaphase. 9. Nucleus in mid-metaphase. Three chromosomes gathering in the central portion of the nucleus are seen as a black dot. 10–18. Corresponding drawings of photomicrographs shown in figs. 1–9, respectively.

division of *Porphyra umbilicalis* (L.) Kütz. var. *laciniata* (Lightf.) J. Ag., Krishnamurthy (1959) states that in early prophase a number of discrete staining bodies are seen in favourable materials, and these are found surrounding a central nucleole which is feebly stained and ill defined. Such event of the feature in early prophase has not been met with in the present species. With careful observation, the feebly stained linnen reticulum which shows relatively course network structure was occasionally observed within the nuclear cavity. The appearance of this reticulum shows an indication to the onset of the prophase stage of the nucleus. In the mid-prophase nucleus in the vegetative cells of *Porphyra*, chromosomes surrounding the nucleolus have been observed by Krishnamurthy (1959) in *P. umbilicalis* var. *laciniata* and by Migita (1967) in *P. yezoensis* and by Yabu (1969) in several species of *Porphyra*. However, in my materials, chromosomes have a marked tendency to make their appearance after the nucleolus had disappeared entirely. Thus, usually for a very short while, the nucleus enters the stage where both nucleolus and chromosomes are obscure within its nuclear cavity. Fig. 1 indicates the nucleus in prophase stage just before the formation of chromosomes begins. In this stage small chromatin granules can be seen within the nuclear cavity. Three chromosomes are soon formed from these granules; at first they are slender like threads and then gradually become short. One of the chromosomes occasionally takes a hook form in early metaphase (Fig. 4). In mid-metaphase chromosomes gather in the central region of the nuclear cavity (Figs. 8–9). The direction of the nuclear division occurs in a parallel but rarely oblique or perpendicular plane (Fig. 8) to the thallus surface. After the completion of the nuclear division, the cell is transversely segmented.

Nuclear division in spermatium formation: Comparing the spermatium mother cell with the vegetative cell, the former is oblong in shape and the latter usually squarish as seen in Figs. 1–21. In the spermatium mother cells, as in the case of the somatic cell division, no marked differences could be noticed in the nucleus between the resting stage and the early prophase, and it was also observed that

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19–31. Various stages of nuclear division in spermatium formation: **19–20.** Nucleus in early metaphase in spermatium mother cells. **21.** Nucleus in the side view of metaphase in spermatium mother cell. **22.** Nucleus in anaphase in spermatium mother cell. **23–24.** Nucleus in late prophase in the second division. **25–27.** Corresponding drawings of the photomicrographs shown in figs. 19–21, respectively. **28–29.** Corresponding drawings of the photomicrographs shown in figs. 23–24, respectively. **30–31.** Nuclei in metaphase in the fourth division.

32–37. Various stages of nuclear division in carpospore formation: **32–33.** Nucleus in early metaphase in carpospore mother cells. **34.** Nucleus in early metaphase in the second division. **35–37.** Corresponding drawings of photomicrographs shown in figs. 32–34, respectively.

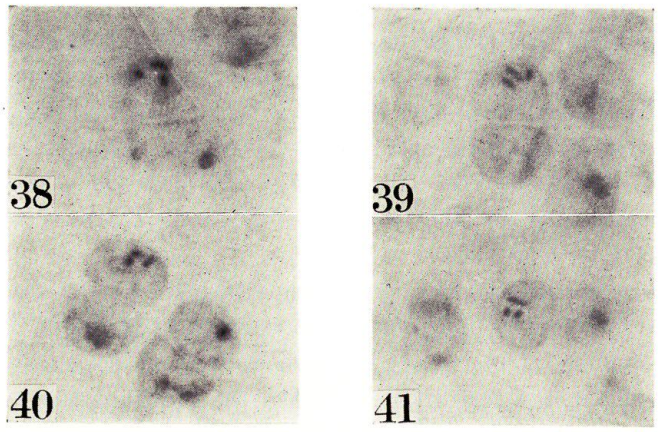
(Magnification. Figs. 1–9, ×1100; Figs. 19–24, & 32–34, ×1,200; Figs. 30–31, ×1,600)

chromosomes usually come into existence after the nucleolus had disappeared. Of course in these cells the number of chromosomes was easily counted to be three (Figs. 19–20). At late prophase each chromosome is not so slender in form as in the vegetative cells, but spherical already, and at metaphase it is larger in size than in vegetative cells. Within the spermatium mother cell, a nuclear division always occurs towards the longitudinal cell axis (Fig. 22), thus the cell was eventually divided perpendiculary to the surface of the thallus. The prophase nucleus from the second to later successive divisions is somewhat peculiar. In those divisions the nucleus at prophase is seen apparently to have a reticulum within the nuclear cavity where the nucleolus had already vanished away. This reticulum increases its staining ability every successive division, so that it attains the strongest staining in the last division. When the reticulum comes out of sight the outline of chromosomes appears. In these successive divisions three chromosomes were also easily counted in nuclei at late prophase and metaphase. In any celled-stage, chromosomes were stained well at metaphase. In the nuclei at prophase in the second and third divisions one of the chromosomes is occasionally observed to be longer than the other two (Fig. 28). After the fourth cell division 16 spermatia are formed within a spermatangium.

Nuclear division in carpospore formation: Intrusion of spermatium and fusion of gamete nuclei could not be ascertained within the carpogonium. However the good figures of nuclei at metaphase seen in the carpogonium showed that all of the nuclei contained six chromosomes. Each of the chromosomes in the carpogonium makes its appearance as a slender rod form in early metaphase (Figs. 32–33). In the following successive cell divisions, six chromosomes were also easily counted in metaphase nuclei (Fig. 37). Thus undoubtedly the nucleus becomes to be diploid within the carpogonium and carpospores contain a nucleus in diploid. After the third cell division eight carpospores are formed within a carpogonium.

2. *Porphyra nereocystis* Anderson

Numerous thalli of this species were obtained from the stipes of two sporophytes of *Nereocystis luetkeana* Post. et Rupr. The materials obtained agree quite well with the description of Hus (1902) and Smith (1940) on *Porphyra nereocystis* Anderson except the length of thalli. They were only ca. 20–30 cm. long and ca. 5 cm. wide in medium size due to young age and only few of them were maturing. In the present species, the resting nucleus in a vegetative cell, and the nucleolus within its nuclear cavity are smaller in size than those of *P. amplissima* as shown in the following table, and it is also noticeable that in



Figs. 38–41, *Porphyra nereocystis* Anderson

38–40. Chromosomes in early metaphase in the third nuclear division leading to spermatium formation. 41. Chromosomes in early metaphase in the fourth nuclear division leading to spermatium formation. (Magnification. 38–41, ×1,200)

any cell whether it is somatic or not, the nuclear membrane, the nuclear cavity and the nucleolus in the resting and prophase stages were always stained

Table 1. Measurement of resting nucleus and its nucleolus in the vegetative cell of medium size of *Porphyra nereocystis* and *P. amplissima*

Species	Cell	Nucleus	Nucleolus
P. nereocystis	35×20μ	9μ	2.5μ
P. amplissima	40×25μ	15μ	4.0μ

more weakly than those of *P. amplissima*. Dividing nuclei were only obtained in the second to the last cell division for spermatium formation. The mitotic process during these cell divisions was quite similar to that of *P. amplissima* in the point that reticulum became visible at prophase and its staining ability somewhat increased every cell division. This reticulum is also stained more weakly than that of *P. amplissima*. In the prophase stage the nucleus with reticulum increases its size rapidly up to nearly the same or a little larger than that of *P. amplissima*. Early metaphase nuclei found in the cell division leading to spermatium formation are shown in Figs. 38–41. As can be seen in the figures chromosomes are three in number and one chromosome is always found to be a little longer than the other two not only in late prophase but also even in metaphase. Regardless of the staining ability of the reticulum in prophase stage of the nucleus, chromosomes in every cell division were always stained well in metaphase.

Summary

Porphyra amplissima (Kjellm.) Setch. et Hus and *P. nereocystis* Anderson obtained from the stipes of *Nereocystis luekteana* (Mert.) Post et Rupr. that were floating in the Bering Sea were treated cytologically. Nuclear divisions in somatic cells and spermatium and carpospore forming cells were observed in *P. amplissima* and those in speramatium forming cells were observed in *P. nereocystis*. The chromosome number in *P. amplissima* was estimated to be 3 in haploid and 6 in diploid which are in accordance with the report by Kito, Yabu & Tokida (1967) and that in *P. nereocystis* estimated to be 3 in haploid. Between both species, clear differences were recognized in the following points. The resting nucleus and its nucleolus in a somatic cell are larger in size in *P. amplissima* than in *P. nereocystis* and the nucleus and nucleolus in resting and prophase stages in any cell are always stained better in *P. amplissima* than in *P. nereocystis*. So far as observed at metaphase the nucleus in spermatium forming cells, three chromosomes are of the same size in *P. amplissima* but one chromosome is longer than the other two in *P. nereocystis*.

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