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Cytological Observations on Two Species of *Myriodesma* (Fucales: Phaeophyta)

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Abstract

Myriodesma tuberosum J. Agardh and *M. integrifolium* Harvey, collected from Australia, were studied cytologically. At prophase of the oogonium nucleus in both *M. tuberosum* and *M. integrifolium*, the diffuse stage follows after open spireme. In *M. tuberosum*, the peculiar spherical bodies referred to as "chromophilous spherules", "refractive granules associated with nucleolus" and "heterogeneous nucleolus" were at times observed at prophase in the oogonium. In both *M. tuberosum* and *M. integrifolium*, the nuclear division after metaphase in the oogonium is very curious. As soon as the nucleus enters early anaphase, it suddenly comes to stain deeply so that the behaviour of the chromosomes hereafter cannot be easily traced. Without direct division of the mother nucleus in the oogonium, the daughter nucleus, with haploid number of chromosomes, is formed within the oospore. The number of chromosomes in *M. tuberosum* was ascertained to be twenty-four in the oogonia and antheridia, and more than thirty in the wall cells of the conceptacles; in *M. integrifolium* the number was four in the oogonia and antheridia and eight in the antheridial hair cells and wall cells of the conceptacles.

Introduction

I had an opportunity to visit Australia on board the training ship "Oshoro Maru" of the Faculty of Fisheries, Hokkaido University, on her 16th voyage (to the Great Australian Bight) and to collect and fix some seaweeds from Australia for cytological study. The present paper gives the cytological results on two species of brown algae belonging to the genus *Myriodesma*, viz., *M. tuberosum* J. Agardh and *M. integrifolium* Harvey, both of which are described by Nizamuddin & Womersley[1].

I am much indebted to Dr. J. Tokida, Emeritus Professor of Hokkaido University, for his permission to go on board the training ship. Thanks are also due to Dr. S. Motoda, Emeritus Professor of Hokkaido University, to Captain T. Fujii and the officers and crew of the training ship "Oshoro Maru" of the Hokkaido University, as well as to Mr. L.R. Thomas of the Western Australia Regional Laboratory, Nelands, W. Australia, for their kind support in collecting the materials for this study. I am very grateful to Dr. H.B.S. Womersley of Adelaide University, South Australia, for his courtesy in identifying the specimens used here and in reading and correcting the manuscript.

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Materials and Methods

The material of *Myriodesma tuberosum* J. Agardh was caught in a trawl fishing net of the training ship "Oshoro Maru" from the sea bed at 33'01"S. and 126'29"E. on December 23, 1965, while that of *M. integrifolium* Harvey was collected on a reef about 70 km north of Fremantle, W. Australia, on November 15, 1965, as well as being caught in a trawl fishing net with the *M. tuberosum* J. Agardh. After collection, they were brought to the laboratory room on the training ship and were kept alive in vats filled with sea-water. Fixing was made during the night with Tahara's solution[2] which is composed of 2% osmic acid 5 ml, stock solution of chromic acid (sea-water 98 ml, saturated water solution of chromic acid 2 ml) 70 ml, glacial acetic acid 2.5 ml and sea-water 30 ml. Twelve hours after fixing of the materials, they were washed with running water for about six hours, and were put into 30%, 50% and 75% alcohol at an interval of thirty minutes. The materials thus preserved in 75% alcohol were taken to the Phycological Laboratory at Hakodate, Faculty of Fisheries, Hokkaido University. Sections were made by the paraffin method at 4–7 μ thickness and were stained with Heidenhain's haematoxylin.

Results

In both *Myriodesma tuberosum* J. Agardh and *M. integrifolium* Harvey, hermophrodite conceptacles are formed on both sides of the thallus (Pl. I, figs. 2 & 4). Within a conceptacle, antheridial hairs develop from the wall cells near the upper part of the conceptacle, while oogonia develop from near the basal part.

1. *Myriodesma tuberosum* J. Agardh

a) *Nuclear division in the oogonium*

The young oogonium is formed as a projection from a wall cell of the conceptacle. A nucleus, at resting stage in a young oogonium, shows a deeply stained nucleolus (about 3 μ in diameter) which has occasionally vacuolated structure. When the nucleus goes into prophase, it grows in size rapidly and when about 20 μ in diameter, the very thin chromatin threads are found to gather at one corner of the nuclear cavity (Pl. II, fig. 5). In this synapsis stage, chromatin threads are feebly stained at first but soon they increase a little in both their staining affinity and their thickness (Pl. II, fig. 6). Plate II, fig. 7 indicates a nucleus in open spireme. After this stage, the nucleus passes through the diffuse stage. In the late diffuse stage, the nucleolus becomes obscure. The diffuse stage is supposed to be fairly long in duration, because this time was found more often than other stages of prophase.

During the period from late synapsis to the end of the diffuse stage, three kinds of peculiar spherical bodies have been found at times, apart from the nucleolus within the nuclear cavity. The first kind of body appears during the period from early synapsis to open spireme; it is always one in number and stains somewhat deeper than the nucleolus (Pl. II, figs. 6 & 7). This body is thought to be identical with the "chromophilous spherule" which had been reported in the tetraspore mother cells in some Dictyotales (Mottier[3]), Williams[4]), Carter[5]), Yabu[6]), Kumagae, Inoh & Nishibayashi[7])) and in the first meiotic division of oogonium and antheridium in some Fucales (Tahara[2]), Okabe[8]), Hiroe & Inoh[9])[10]), Inoh & Hiroe[11])[12])). The second kind of body appears mostly in the diffuse stage, being very variable in number (2–20) and in size, (more or less than 1 μ) and they usually gather around the nucleolus (Pl. II, figs. 8–13; Pl. III, figs. 14–15). This body is supposed to be the same as the "refractive granules associated with nucleolus" which were found in prophase of *Halidrys siliquosa* by Moss & Eilliot[13]) who said that they resembled very much the figures of "intra nucleolar chromosomes" described by Svedelius[14]) in the red alga *Lomentaria rosea*. According to Svedelius[14]) these granules in *Lomentaria rosea* afterwards enter the nucleolus, but such an event could not be observed in the present species. The third kind of body appears during the period from synapsis to the diffuse stage, being always one in number, about 3 μ diameter (nearly the same size as the nucleolus), but it stains uniformly weak as can be seen in the nucleus shown in Pl. II, fig. 13 & Pl. III, fig. 14. This is thought to be the "heterogeneous nucleolus".

In diakinesis, the chromosomes appear first as faintly stained thin chromatin threads spreading all over the nuclear cavity. These slender chromosomes soon become thick and short, and then they gradually gather near the center of the nucleus. At metaphase, 24 chromosomes were usually counted. At the side view of metaphase, faintly stained spindles were recognized (Pl. III, fig. 20). The later stages of nuclear division are very peculiar. As soon as a nucleus enters early anaphase, it suddenly comes to show a strong affinity for the staining solution, probably due to a rapid physiological change in karyolymph, so that the behaviour of chromosomes could not be traced hereafter. At the same time the area surrounding a nucleus comes to be filled with dense cytoplasm. After a while, the nucleus (which is seen as a dark large body) goes into the resting stage with a nucleolus, and then recovers normal staining affinity.

Among the numerous oogonia in the preparations, a few were found to contain two daughter nuclei. One of the oogonia is shown in Pl. III, fig. 21. Those daughter nuclei are supposed to be formed in the normal process of the nuclear division after metaphase, but the formation of these two nuclei in oogonia should be regarded as abnormal in the present species.

b) *Nuclear division in the antheridium*

Dividing nuclei were encountered in numerous antheridia, but good metaphase, in which the chromosome count was possible, were met only in some of the first and second nuclear divisions in antheridia. About 24 chromosomes were observed in them. In the side view of metaphase, deeply stained spindles were noticed as shown in Pl. III, fig. 22, but a centrosome was not recognized at the pole.

c) *Nuclear division in somatic cells*

Although somatic nuclear divisions were occasionally observed in the hair cells of conceptacles, counts of chromosomes were not possible in any metaphase nucleus. However, some metaphase nuclei clearly has more than thirty chromosomes.

2. *Myriodesma integrifolium* Harvey

a) *Nuclear division in the oogonium*

The nucleus in early prophase enlarges rapidly and comes to have small chromatin granules in its nuclear cavity. Soon chromatin threads develop from these granules. During the course of the nuclear division in the oogonium, a nucleus attains maximum size in spireme. The three kinds of peculiar spherical bodies, which were seen at times in the nucleus at prophase in the oogonium of *M. tuberosum* could not be found in the present species. Similarly to *M. tuberosum*, the diffuse stage follows after an open spireme. When the nucleus enters the diffuse stage, dense cytoplasm comes to surround the nucleus. In the diffuse stage, the chromatin threads spread over the nuclear cavity as a coarse network. When the nucleus enters diakinesis, the chromatin threads are still visible and the chromosomes begin to appear as if they were knots on the threads of chromatin. At diakinesis, the nucleolus disappears completely in *Myriodesma tuberosum* but it occasionally remains in the present species. The chromosomes are found to be four in number, two being somewhat larger than the other two. The largest chromosome is nearly the same size as the nucleolus, so that if the nucleolus still remains within the nucleus it is apt to be confused with the chromosomes. As in *M. tuberosum*, the subsequent stages of division after metaphase are also very curious. In many cases, both the nucleus and the chromosomes within it at near mid-metaphase become small in size and then the chromosomes come to gather at the center of the nucleus as seen in Pl. V, fig. 34, where the nuclear membrane vanishes completely. After this, the nucleus (which begins to elongate) comes to stain well. At the same time the chromosomes gathering at the center of the nucleus become well stained and dot-like (Pl. V, fig. 35). After a while, the nucleus grows larger and rounded, with an increase of staining affinity, and at last the content within the nuclear cavity cannot be discriminated due to its deep staining. In some cases, the nucleus becomes heavily stained well before the stage of mid-metaphase (as seen in Pl. V, figs. 32 & 33), and then it turns directly to the stage of Pl. V, fig. 37 where four chromosomes, nearly the same size as in the nucleus of

Pl. V. fig. 32, were still barely visible. A well stained large globular nucleus like this, soon decreases in its staining affinity and the nucleolus begins to appear within the nuclear cavity. The staining condition of the nucleus becomes gradually normal, and it is then the daughter nucleus in the resting stage of the egg as seen in Pl. V, fig. 40. The oogonium with two daughter nuclei, which is supposed to be abnormal, is also sometimes observed as in *M. tuberosum*.

b) *Nuclear division in the antheridium*

In the first division of the antheridium, few are found in synapsis or in spireme and none in diakinesis, but many in metaphase. Four chromosomes were always recognized on the polar view of metaphase (Pl. VI, fig. 44). In the side view of metaphase, spindle fibres were observed, but centrosome could not be seen at the pole. Numerous metaphase nuclei were found also in the following successive nuclear divisions. At metaphase of those divisions, four chromosomes were easily counted (Pl. VI, figs. 45–48).

c) *Nuclear division in somatic cells*

Dividing nuclei in the somatic cells were observed in the wall cells of conceptacles and in the antheridial hair cells. From the metaphase of those divisions, the chromosomes in somatic cells were ascertained to be eight in number (Pl. VI, figs. 41–43).

Discussion

Since 1896 when Farmer & Williams[15)] first published on nuclear division in the species of Fucales, cytological investigations in that order have been made by many workers, but such study in the genus *Myriodesma* was only made by Nizamuddin[16)] in 1962, who described the resting nucleus in the somatic and reproductive cells or in the embryonal development in *M. integrifolium*, *M. latifolium* and *M. quercifolium*. Among these previous investigations, the report on *Halidrys siliquosa* by Moss & Elliot[13)] is peculiar in so far as the reduction division was suggested to occur in the germination of oospore, however the work of Moss & Eilliot was proved incorrect by Naylor[17)18)]. The present study on *Myriodesma tuberosum* and *M. integrifolium* reveals that the reduction division takes place in the formation of the oospores and sperms, as reported in many previous cytological works on Fucales.

Between *Myriodesma tuberosum* and *M. integrifolium*, some disparities were observed in meiosis of the oogonium as follows: The prophase nucleus is found at times to have "chromophilous spherule", or "refractive granules associated with nucleolus" or "heterogeneous nucleolus" in *M. tuberosum* but none in *M. integrifolium*. At diakinesis, the nucleolus disappears completely in *M. tuberosum* but it occasionally remains in *M. integrifolium*.

In both *M. tuberosum* and *M. integrifolium*, the diffuse stage, which is of fairly long duration, follows after open spireme. The diffuse stage had been observed in several species of Dicytotales, such as *Dictyota dichotoma* (Williams[4]; Kumagae, Inoh & Nishibayashi[7]), *Padina pavonia* (Carter[5]) and *P. japonica* (Kumagae, Inoh & Nishibayashi[7]), but it has not previously been observed in any species of Fucales. In the presence of this diffuse stage, the two species of *Myriodesma* studied here seem to be somewhat more related to the Dictyotales than the Fucales.

It is well known that many of the Fucales give rise to eight nuclei in the oogonia as the result of the three successive nuclear divisions. In both *Myriodesma tuberosum* and *M. integrifolium* however, the behaviour of the nucleus after metaphase of the first division in the oogonium is very curious and it was noticed that the mother nucleus of the oogonium became a daughter nucleus without a direct division. Probably at anaphase of the mother nucleus in the oogonium, one group of daughter chromosomes which were separated towards the pole, soon ceases to exist within the nucleus, and only the other group of chromosomes comes to contribute to the nucleus in the egg.

References

1) Nizamuddin, M. and Womersley, H.B.S. (1966). The morphology and taxonomy of *Myriodesma* (Fucales) *Nova Hedwigia* 12, 373–383.

2) Tahara, M. (1929). Ovogenesis in *Coccophora Langsdorfii* (Turn.) Grev. *Sci. Rep. Tohoku Imp. Univ.* 4th Ser. IV, 551–556.

3) Mottier, D.M. (1900). Nuclear and cell division in *Dictyota dichotoma*. *Ann. Bot.* 14, 163–192.

4) Williams, J.L. (1904). Studies in Dictyotaceae 1. The cytology of the tetrasporangium and the germinating tetraspore. *Ibid.* 18, 141–158.

5) Carter, P.W. (1927). The life-history of *Padina pavonia*. 1. The structure and cytology of the tetrasporangial plant. *Ibid.* 41, 139–159.

6) Yabu, H. (1958). On the nuclear division in tetrapsorangia of *Dictyopteris divaricata* (Okam.) Okam. and *Dictyota dichotoma* Lamour. *Bull. Fac. Fish. Hokkaido Univ.* 8, 290–296.

7) Kumagae, N., Inoh, S. and Nishibayashi, T. (1960). Morphogenesis in Dictyotales II. On the meiosis of the tetraspore mother cell in *Dictyota dichotoma* (Hudson) Lamouroux and *Padina japonica* Yamada. *Biol. Jour. Okayama Univ.* 6, 91–102.

8) Okabe, S. (1929). Meiosis in Oogonium von *Sargassum Horneri* C. Ag. *Sci. Rep. Tohoku Imp. Univ.* 4th Ser. V, 661–669.

9) Hiroe, M. and Inoh, S. (1954). Cytological studies on the Fucaceous plants IV. On the mitotic division in the antheridium of *Sargassum Horneri* (Turn.) Ag. (Preliminary note). *Bot. Mag.* Tokyo 67, 190–192.

10) Hiroe, M. and Inoh, S. (1956). Cytological studies on the Fucaceous plants VI. On the meiotic division in the antheridium of *Sargassum tortile* C. Ag. (a preliminary note), *La Kromosomo* 27–28, 942–947.

11) Inoh, S. and Hiroe, M. (1954). Cytological studies on the Fucaceous plants II. On the meiotic division in the antheridium of *Hizikia fusiformis* Okamura (a preliminary note). *Ibid.* 21, 764–766.

12) Inoh, S. and Hiroe, M. (1964). The cytological study of the Fucales plants III. On the meiotic division in the antheridium of *Sargassum piluliferum* A. Ag. (a preliminary note). *Ibid.* **27–28**, 942–947.
13) Moss, B and Elliot, E. (1957). Observations on the cytology of *Halidrys siliquosa* (L.) Lyngb. *Ann. Bot.* N.S. **21**, 143–151.
14) Svedelius, N.E. (1937). The apomeiotic tetrad division in *Lomentaria rosea* in comparison with the normal development in *Lomentaria clavellosa*. *Symbolae Bot. Upsaliensis* **3**, 3–54.
15) Farmer, J. B. and Williams, J. L. (1896). Contributions to our knowledge of the Fucaceae, their life-history and cytology. *Phil. Trans. Roy. Soc. Lond. B.* **190**, 623–645.
16) Nizamuddin, M. (1962). Structure and development of *Myriodesma* (Fucales). *Bot. Gaz.* **124**, 68–74.
17) Naylor, M. (1958). Chromosomes and chromocenters in the nuclei of *Halidrys siliquosa* (L.) Lyngb. *Nature* **181**, 853.
18) Naylor, M. (1958). The cytology of *Halidrys siliquosa* (L.) Lyngb. *Ann. Bot.* N.S. **22**, 205–217.

Explanation of Plates

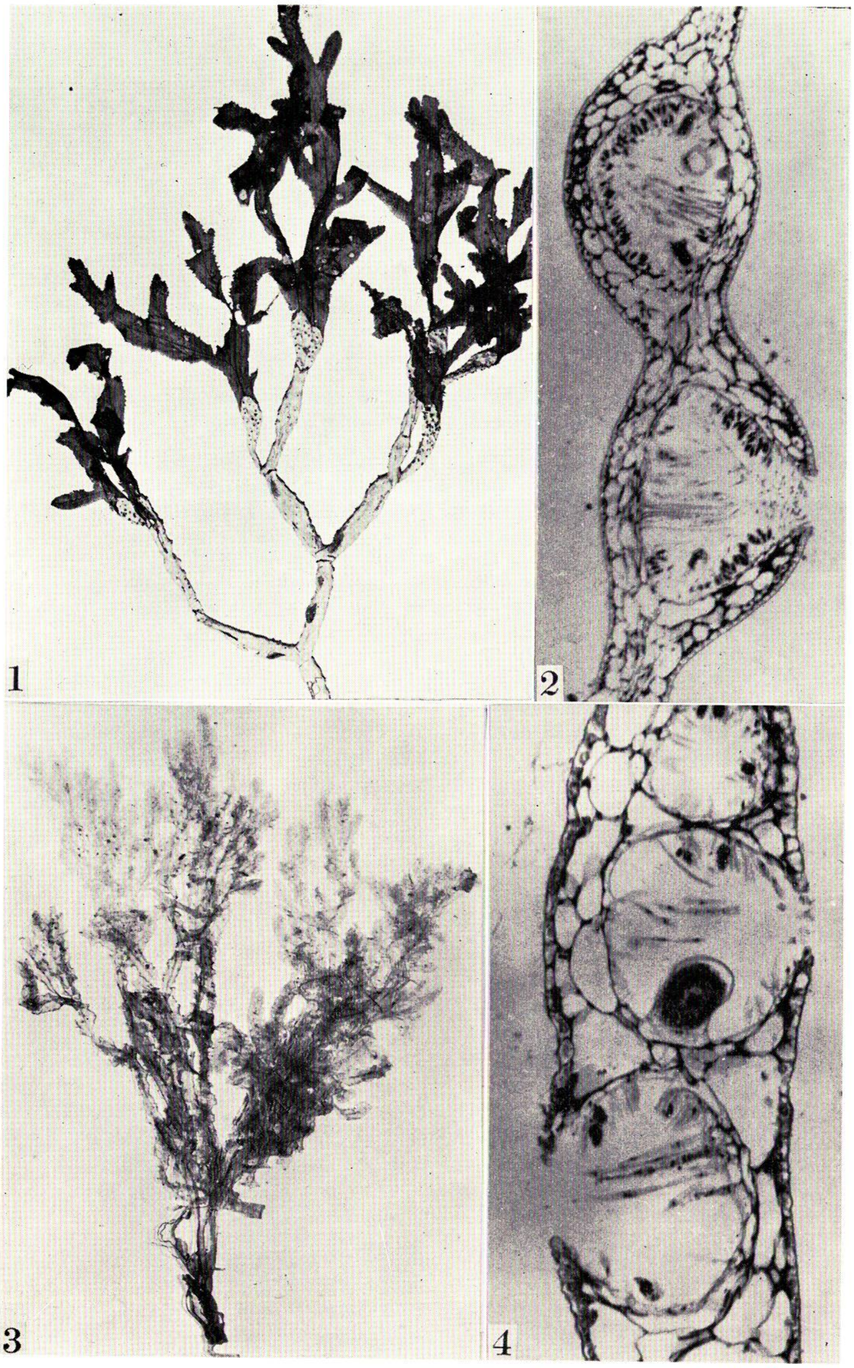
PLATE I

Myriodesma tuberosum J. Agardh

1. Habit of portion of thallus. (×1/2)
2. Cross section of a part of thallus through conceptacle. (×120)

Myriodesma integrifolium Harvey

3. Habit of thallus. (×3/4)
4. Cross section of a part of thallus through conceptacle. (×150)



YABU: Cytological observations on *Myriodesma*

PLATE II

Myriodesma tuberosum J. Agardh

Various stages of nuclear division in the oogonium.

5. Nucleus in early synapsis. Thin and faintly stained chromatin threads, which are connected with the nucleolus, are seen at the corner of the nuclear cavity.

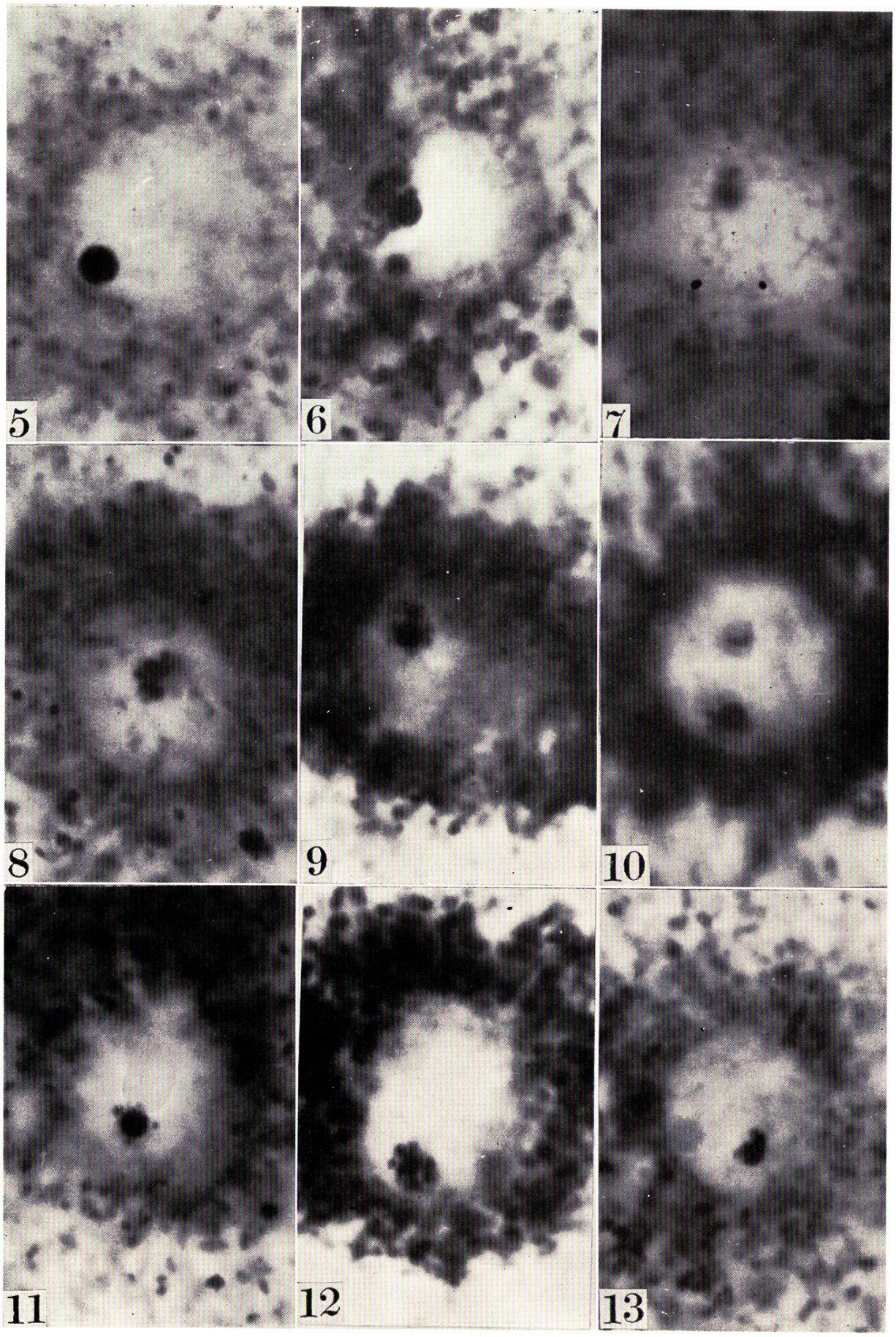
6. Nucleus in synapsis. Chromophilous spherule is seen within the nuclear cavity.

7. Nucleus in open spireme. Chromophilous spherule is seen in the upper side of the nucleolus.

8. A later stage than fig. 7. The chromatin threads begin to fade away, commencing the diffuse stage of the nucleus. The refractive granules associated with the nucleolus are visible near the center of the nuclear cavity.

9–13. Nucleus in the diffuse stage. The refractive granules associated with the nucleolus are seen within the nuclear cavity. In fig. 13, the heterogeneous nucleolus is visible at the corner of the nuclear cavity.

(All figures, $\times 1,600$)



YABU: Cytological observations on *Myriodesma*

PLATE III

Myriodesma tuberosum J. Agardh

Various stages of nuclear division in the oogonium.

14–15. Nucleus in the diffuse stage. Heterogeneous nucleolus and the refractive granules associated with the nucleolus are seen within the nuclear cavity. Fig. 15 is focussed to the refractive granules and the heterogeneous nucleolus is not visible in this figure.

16. Nucleus in late diffuse stage. The nucleolus has disappeared completely.

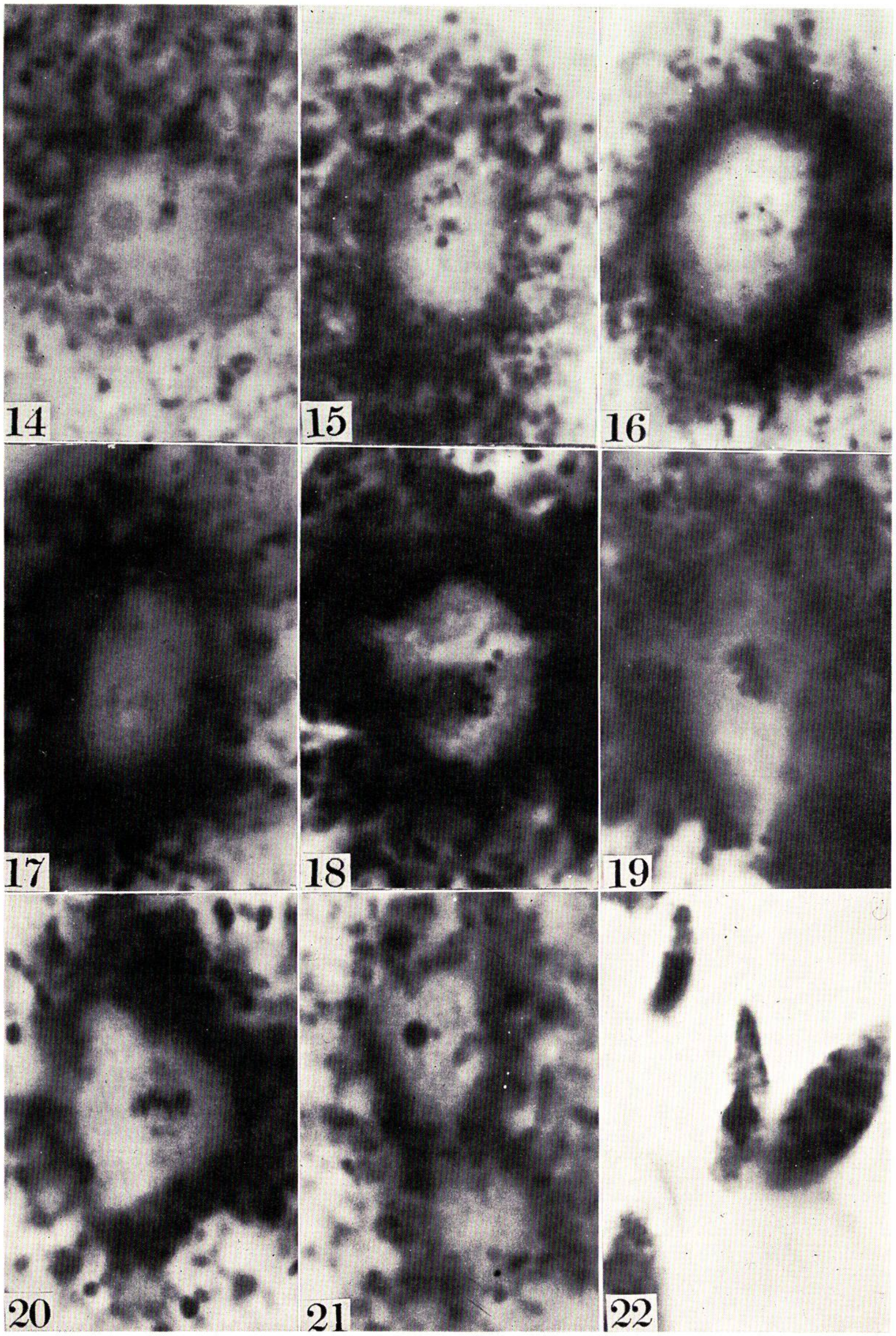
17. Nucleus in early diakinesis.

18–19. Nucleus in metaphase.

20. Side view of metaphase.

21. Two daughter nuclei in the resting stage. The focus is brought to the nucleus in one of the two nuclei lying above and below.

22. Side view of metaphase in the first nuclear division in the antheridium.
(All figures, $\times 1{,}600$)



YABU: Oytological observations on *Myriodesma*

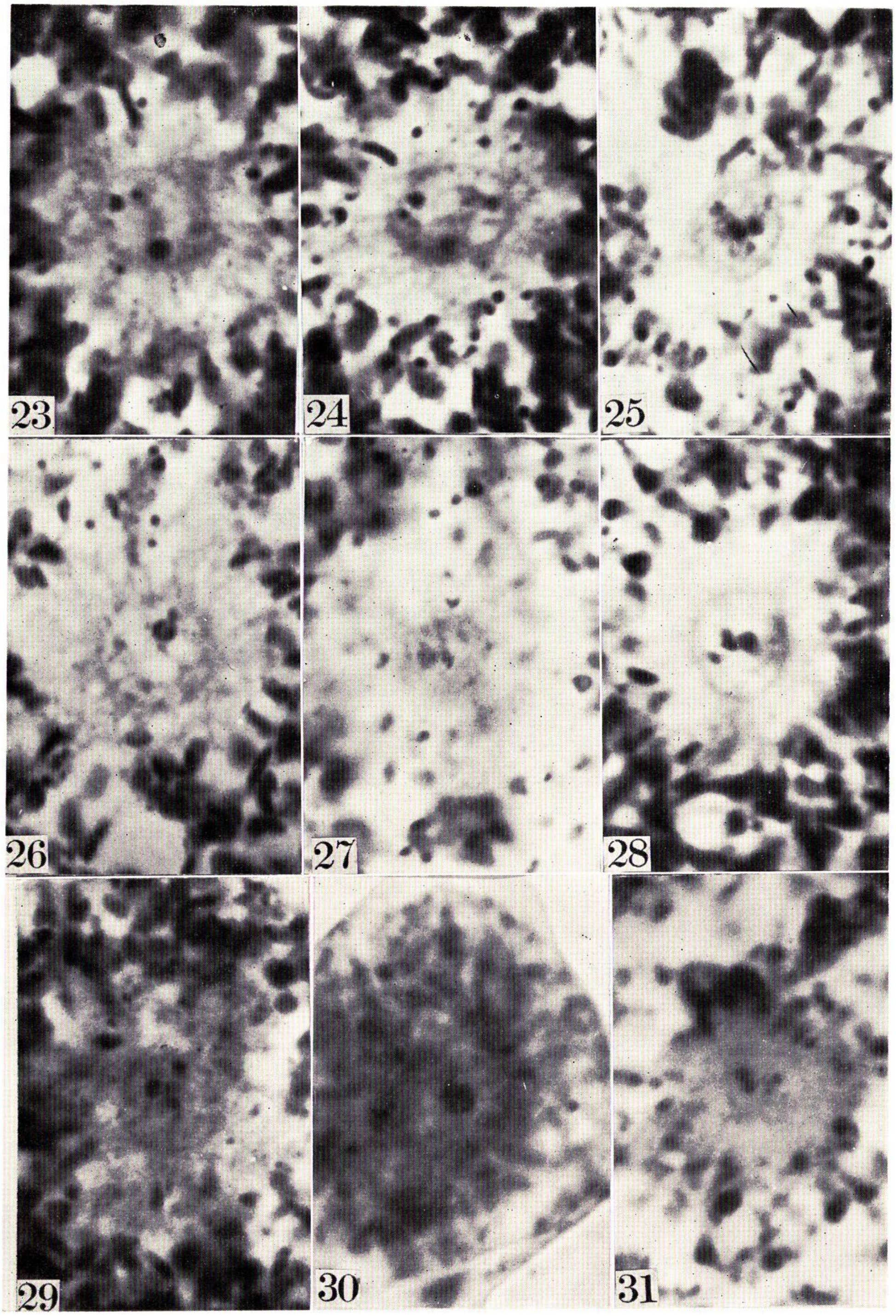
PLATE IV

Myriodesma integrifolium Harvey

Various stages of nuclear division in the oogonium.

23–28. Nucleus in late diakinesis.

29–31. Nucleus in metaphase.
(All figures, ×1,600)



YABU: Cytological observations on *Myriodesma*

PLATE V

Myriodesma integrifolium Harvey

Various stages of nuclear division in the oogonium.

32–33. Nucleus in metaphase.

34. Nucleus in the side view of metaphase.

35. Nucleus in a later stage than fig. 34. Assembled chromosomes are seen as a small dot at the center of the elongated nucleus.

36. Nucleus in a later stage than fig. 35. Nucleus becomes deep in staining. Hereafter, the nucleus grows larger in size and stains more deeply as in fig. 37.

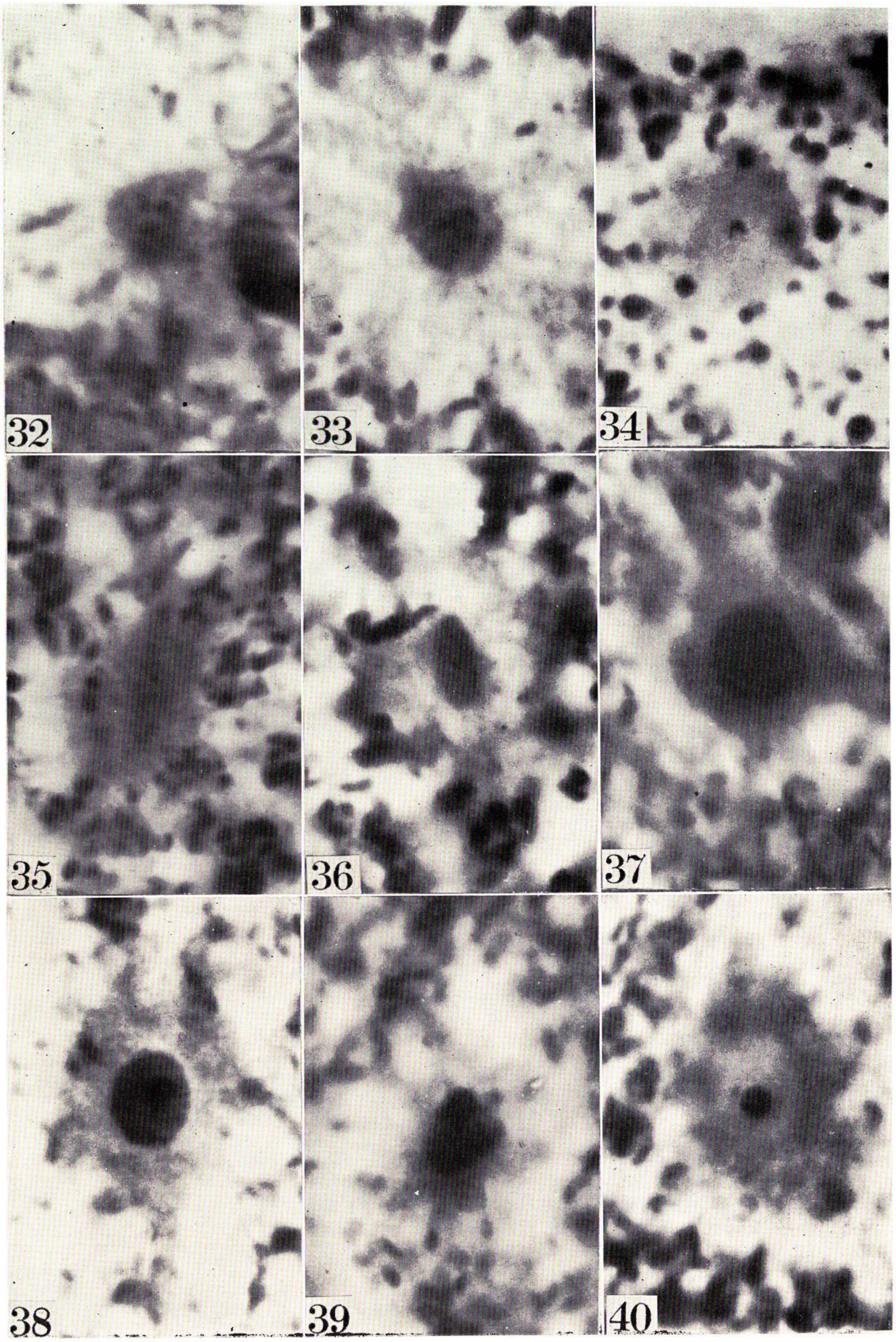
37. Nucleus in a later stage than in figs. 32–34. The nucleus comes to be stained deeply and at the same time cytoplasm surrounding the nucleus becomes more condensed. Within the nucleus of this figure, four chromosomes, which are nearly the same size as those seen in fig. 32, are still barely observed, but do not show well in this photograph.

38. Nucleus in a later stage than fig. 37.

39. Nucleus in a later stage than fig. 38.

40. Resting stage of daughter nucleus which is formed after completion of nuclear division in the oogonium.

(All figures, $\times 1,600$)



YABU: Cytological observations on *Myriodesma*

PLATE VI

Myriodesma integrifolium Harvey

41. Part of a hair cell in a conceptacle. A nucleus in various stages is seen in each cell.

42. Nucleus in late prophase in the basal cell of an antheridial hair.

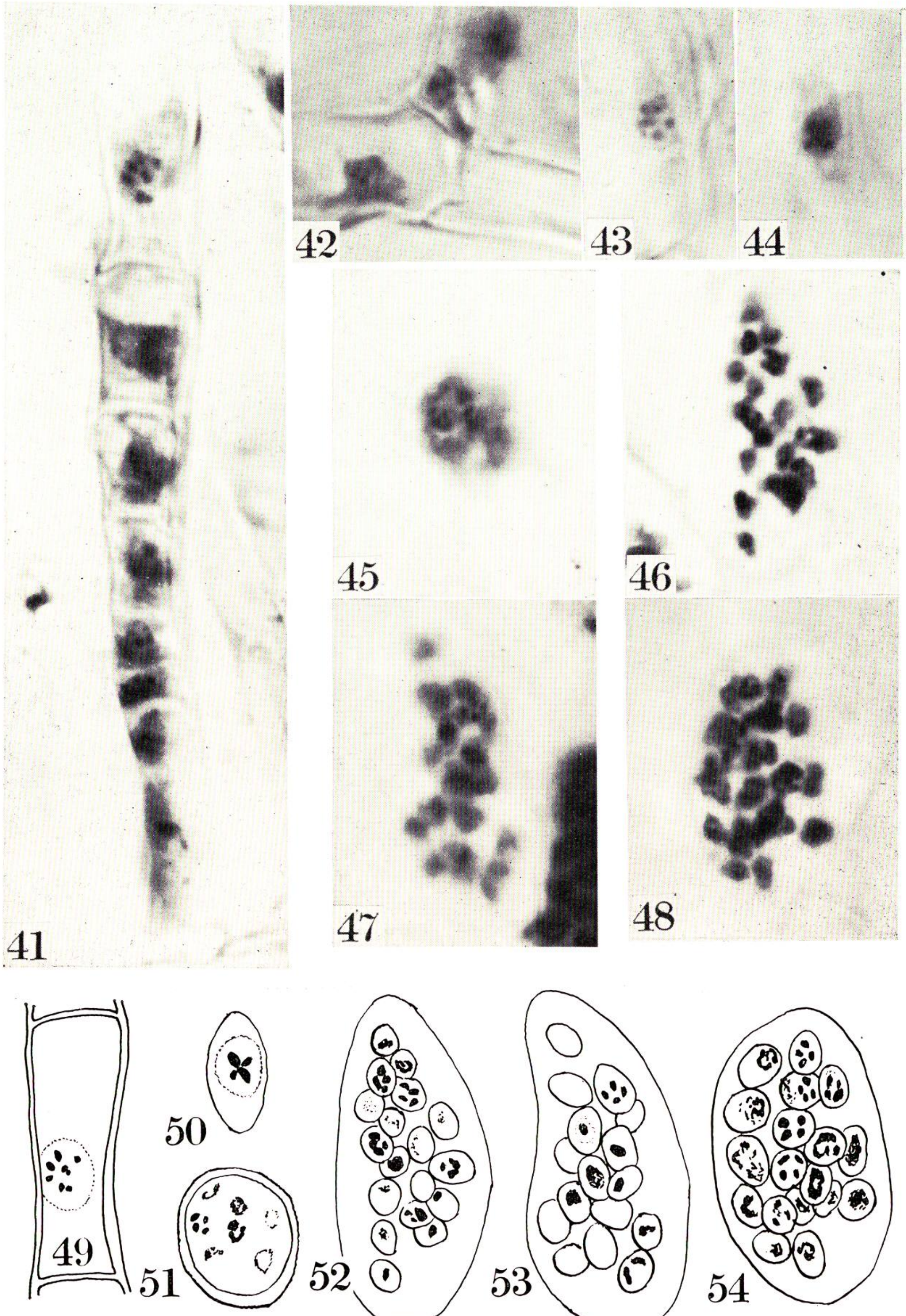
43. Nucleus in metaphase in a wall cell of a conceptacle.

44. Nuclues in metaphase in the first nuclear division in an antheridium.

45–48. Various stages of nuclear divisions leading to spermatozoid formation in antheridia.

49. Corresponding drawing of the uppermost cell in the hair of the conceptacle shown in fig. 41.

50–54. Corresponding drawings of nuclear division in the antheridia shown in figs. 44–48, respectively.
(All figures, $\times 1,600$)



YABU: Cytological observations on *Myriodesma*