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On the Life-History of *Anadyomene wrightii* with Special Reference to the Reproduction, Development, and Cytological Sequences*

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Abstract

The sexual and asexual reproduction, development and cytology of a marine alga, *Anadyomene wrightii* have been studied by culture experiments. The plants produced and liberated swarmers at 23°, 2500 lux, 14 hr light, 10 hr dark condition. There are three types of plants; one is sporophyte, producing quadriflagellate zoospores and the other two are male and female gametophytes, forming biflagellate isogametes. These three plants are superficially similar and can not be distinguished from one another. The gametes from the same thallus do not fuse, but the copulation takes place between those from thalli of different sex. The mode of the germination of zoospores is similar to those of the zygotes. It is of the so-called "Typus filamentosus immediatus", showing bipolar nature. The method of septation of the germlings is not of the segregation, but of the centripetal formation. The somatic chromosome number of germlings derived from the zoospores is 12–15, and those derived from the zygotes is 25–30. The shape of zoospore and gamete, the mode of germination, the type of the alternation of generations and the manner of septum formation, all support the circumscription that the Anadyomenaceae is a taxon belonging to the Cladophorales.

Introduction

Concerning the reproduction and the development of the genus *Anadyomene*, little is known so far. Derbès et Solier[1] gave some figures and statements about *A. stellata*. They observed "sporozoides" formed in the cells of the thallus, but did not give details of the nature of these sporozoides. Several authors such as De Toni[2], Collins[3], Wille[4], Printz[5] and Fritsch[6] dealt with the reproduction of the present genus after Derbès et Solier's findings, but did not add any further information. In 1940, Iyengar and Ramanathan[7] described the reproduction of *A. stellata*. It was the first which gave details to some extent regarding the reproduction of the present genus. They observed both asexual reproduction by means of quadriflagellate zoospores and sexual reproduction by means of biflagellate gametes which were isogamous, and as a result, they speculated about the existence of the isomorphic generations. However, they did not give any figure on it. Recently Jónsson[8] reported the zoospore formation and its germination with *A. stellata*. Phillips[9] described the development of the young thallus of the same species from the field, but he did not observe any reproductive cells.

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As for *Anadyomene wrightii*, Heydrich[10] made some observations on the formation of the "Zwischenzelle" (intervening cell) and mentioned that the "Zwischenzelle" was formed by means of cell division of "Hauptzelle" (axial cell) and sometimes it was formed by means of germination of "Akinete" which attached itself to the "Hauptzelle". However, detail of the formation and the origin of this "Akinete" were not described. In 1908, Okamura[11] made the similar observation as Heydrich's, and supported Heydrich's opinion. According to Yamada and Chihara (private communications), they observed the liberation of swarmers of the present alga in their natural environment off Okinawa Island and Hachijo Island of Japan.

During the past several years we have cultured the vegetative thalli of *Anadyomene wrightii* and succeeded in obtaining swarmers liberated from them. The present paper deals with the gamete- and zoospore-formations, the developments of both zygotes and zoospores, the septum formations, some cytological observations and some remarks on its systematic position.

Before going further, we wish to express our sincere thanks to Dr. M. Chihara and Dr. Y. Yamada who gave us valuable suggestions in the course of the present study. We also offer our hearty thanks to Mr. T. Konno and Miss M. Satomi who gave us many copies of the literature concerned. Furthermore we wish to express our sincere thanks to Dr. Y. Nakamura, Dr. M. Tatewaki and Mr. H. Nakahara for their kind guidance in staining technique.

Materials and Methods

The materials of the present study were collected by diving in the sea around Cape Ashizuri at the southwestern terminal of Shikoku Island, Japan (Fig. 1-1). The divings were carried out in April and May from 1966 to 1969. The materials which were kept around 13° in an ice-box were brought to the laboratory at Iwaya on Awaji Island for the observation. After being carefully washed with filtered (Hyflo Super-Cel of 25 mm thick layer) and sterilized (30 min. autoclaved at 120°) sea-water, algal thalli were put one by one into glass vessels which contained 500 m*l* of sterilized and filtered sea-water. No nutriment was added. These vessels were kept under three different conditions, as follows:

No. 1 Light 2500 lux: 14 hr light and 10 hr dark; Temperature 23°±1°.
No. 2 Light 2500 lux: 14 hr light and 10 hr dark; Temperature 18°±1°.
No. 3 In a laboratory room of 2500 lux at maxium under natural indirect sunlight: approximately 16 hr light and 8hr dark; Temperature about 20°-25°.

Toshiba fluorescent lamp DSDL (natural sunlight type) was used as an artificial light source. A quantity of swarmers of both zoospores and gametes were easily obtained through their clearly positive phototactic nature. After zoospores were washed twice with sterilized and filtered sea-water, they were transplanted onto glass blocks (20×20×2 mm). Such glass blocks were kept under dark conditions for one to three hours, while the swarmers were scattered evenly and fixed on the substratum. Then the glass blocks were washed with sterilized sea-water and transferred into glass vessels that contained 20 m*l* of Provasoli's ES-medium. On the other hand, washed zoospores were isolated singly with sterilized capillary pipettes and transplanted directly into a glass tube which contained 20 m*l* of the same medium. As to gametes, after they were washed twice, the sexually different gametes were mixed on a hollow glass slide. Then the zygotes were isolated with sterilized capillary pipettes by utilizing their clearly negative phototactic res-

ponse. Then the zygotes were transplanted onto glass blocks or into glass tubes.

The culture of sporelings was carried out at 23°±1°, and illuminated for 16 hours daily under the 2500-7400 lux of fluorescent lamp (Toshiba DSDL). After about 200 days some cultures were transferred from 23°±1° to 20°±1°, and kept under illumination of 10 hours a day. All cultures were not axenic, but in strictly unialgal conditions.

For the cytological observation, Wittmann's method[12] and Yabu-Tokida's method[13] were applied. Thalli and sporelings were fixed between 10 p. m. and 1 a. m. The fixative was exchanged every day until the green color of the materials completely faded away. Then the materials were stained with aceto-iron-haematoxylin-chloral hydrate. Swarmers were stained with 1% water solution of Gentian Violet after being fixed with 1% osmic acid solution.

Observations and Results

1. Maturation of thallus: When the vegetative thalli were cultured under the above mentioned conditions, No. 1 (at 23°±1°) and No. 3 (20°-25°), the thalli matured and began to liberate swarmers after 20-30 days from the start of the culture. The maturity appeared first at the laterals of the peripheral portion of a frond, and gradually expanded towards the central part. The basal part of a frond remained in a vegetative state. On the contrary, under condition No. 2 (18°±1°); all the thalli remained sterile and began to change color and finally bleached. This experiment shows that the present alga is able to mature at 23°, but not at 18°. The condition appears to be too low to induce the fertility.

2. Swarmer formation: During the course of both gamete and zoospore formation, the initiation of maturity is superficially recognized first of all by a formation of short conical outgrowth, that later becomes a liberation tube (Fig. 1-2, 3). In the subsequent stage, the contents of a vegetative cell become dark, forming a net-work of chloroplasts (Fig. 1-3). The net-work breaks down into a dark green, granular mass; that means the completion of swarmer formation. The vegetative cells of all parts of the thallus, except the basal portion, are capable of converting into reproductive organs.

3. Liberation of swarmers: A pore is opened for liberation at the apex of the above mentioned outgrowth. In the mean time, swarmers that stand still when they fill the mother cell cavity (Fig. 1-5), suddenly begin to move actively. The swarmers finally escape through a pore, one by one (Fig. 1-6, 7). The pores are circular in the surface view and measured 4.0-5.0μ in diameter (Fig. 1-4, 8, 9). The number of swarmers produced in a mother cell totals from two to three hundred but can not be counted exactly. The discharge of all swarmers in a mother cell takes 3-5-10 minutes. Swarmers pass through the liberation pore either with their posterior or anterior towards the outside. The liberated swarmers swim away as soon as they pass through a pore and never form a cluster near a liberation pore. The swarmer liberation generally begins after about six hours illumination. The swarmer liberation of one frond continues for 3-5 hours. The part of the frond where swarmers have been discharged becomes colorless and finally melts away. The cell wall of the swarmer mother cell is striped finely and vertically as often seen in most members of the Cladophoraceae.

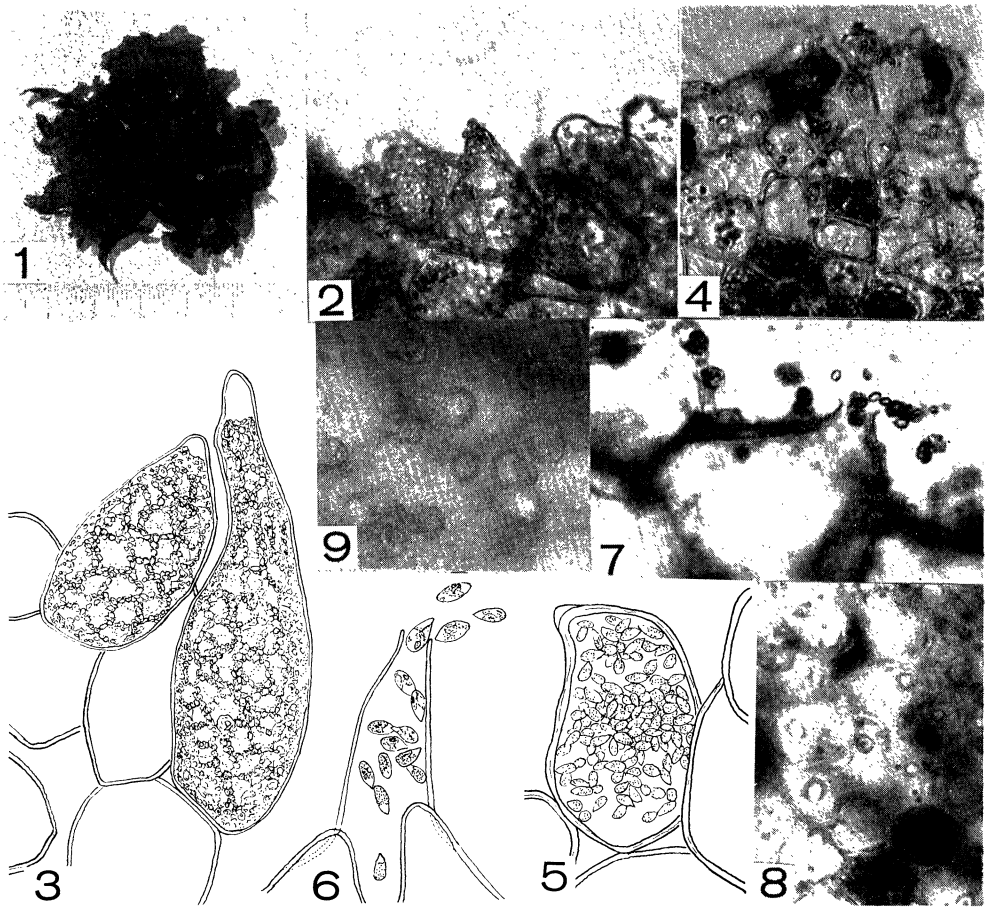

Fig. 1.

There are two types of thalli in the present alga; one produces quadriflagellate zoospores and another produces biflagellate isogametes.* Sexual thalli are heterothallic. There is no difference morphologically between sexual and asexual thalli. Data concerned with the morphological differences among the three kinds of swarmers are comparably summarized in the Table 1.

4. Zoospores: Zoospores are fusiform or teardrop in shape. It contains a chloroplast which is parietal laminate and a stigma which is rod-shaped, colored with light reddish yellow (Fig. 2-1, 2, 3). The liberated zoospores swim vividly and exhibit a slightly positive phototactic response; however, their movements are quite irregular. After they settle on a substratum with the anterior ends of their flagella, they become spherical and are surrounded with a distinct cell membrane within several hours (Fig. 3-1).

5. Gametes and Planozygotes: The morphology of gametes differs from that of

* Isogamy of *A. stellata* was reported by Iyengar and Ramanathan[7].

Table 1. Comparison of characteristics of swarmers.

Structure \ Swarmer	Zoospore	Gamete		Planozygote
		male	female	
number of flagella	4	2	2	4
length of flagella	13.0–15.0μ	14.0–16.0μ	14.0–16.0μ	14.0–16.0μ
number of stigma	1	1	1	2
number of chloroplast	1	1	1	2
number of pyrenoid	1	1	1	2
papillum	+	+	+	+
breadth of body	5.0–.6.5μ	4.0–6.0μ	4.0–6.0μ	7.0–8.0μ
length of body	12.5–16.0μ	10.0–12.0μ	10.0–12.0μ	9.0–12.0μ
phototaxis	+	+	+	–
color of mass	yellowish green	green	green	green

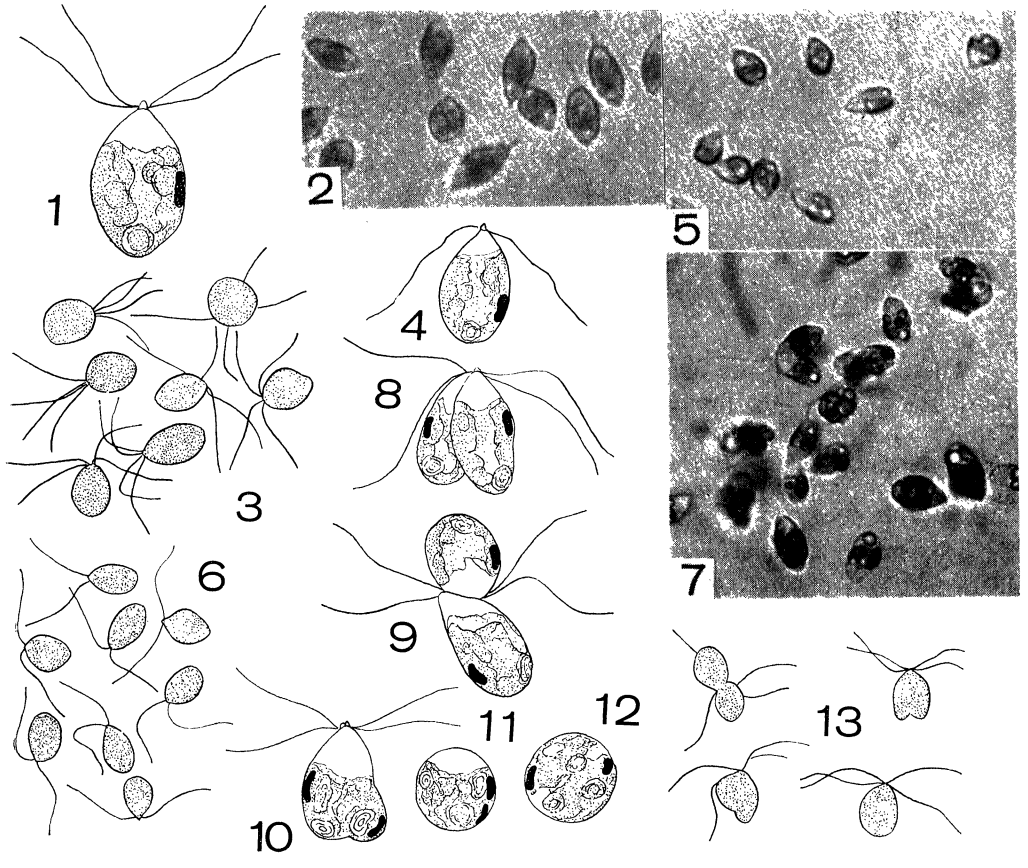

Fig. 2.

zoospores by a slightly narrow breadth, shorter length and the number of flagella. It contains a parietal laminate chloroplast and a stigma (Fig. 2-4, 5, 6).

When the gametes of different sex are mixed, clumping takes place at first, but soon the aggregation of swarmers are disolved and the copulation leads to the formation of planozygotes (Fig. 2-7, 8, ...13). The gametic fusion takes place at first anterior to anterior, affecting gradually towards lateral sides. Gametes derived from the same frond do not copulate. Therefore, the present alga seems to be heterothallic (dioecious). The response of the planozygotes to light slightly negative. The movement of planozygotes soon becomes slower and finally ceases adhering by the apex of a planozygote and its flagella to a substratum. After being deprived of flagella the zygote becomes spherical (Fig. 2-11, 12) soon with a cell wall around themselves (Fig. 3-1).

6. Germination of both zygotes and zoospores: Both zygotes and zoospores germinate immediately after they came to a standstill. No period of dormancy was observed for these. Both zygotes and zoospores are also fundamentally identical with each other in regard to their developmental sequences, such as the type of germination, the shape of germlings, the speed of growth and the type of septum formation.

A stigma of a standstill zoospore or two stigmata of a zygote remained for about 13 days in culture. A germination tube is issued toward an opposite side to the light (Fig. 3-2, 3, ...7 and Fig. 5-2, 3, ...9). Cell contents remain within an original cell. The method of germination is of the so-called Ulva-lactuca type of Chihara[14]; that is the immediate filamentous type of Inoh[15] (Fig. 3-2, 3, 4).

After 7 days the germlings become multinucleate and their chloroplasts become almost laminate, furnished with many pyrenoids; the stigma still remains in the germlings (Fig. 3-5, 6). After 7-9 days they become clavate, with a filamentous rhizoid produced by continuous elongation of the germination tube (Fig. 3-5, 6, 7) (Fig. 5-6, 7, 8, 9). These germlings develope into a long, non-septate siphonous plant (Fig. 3-7

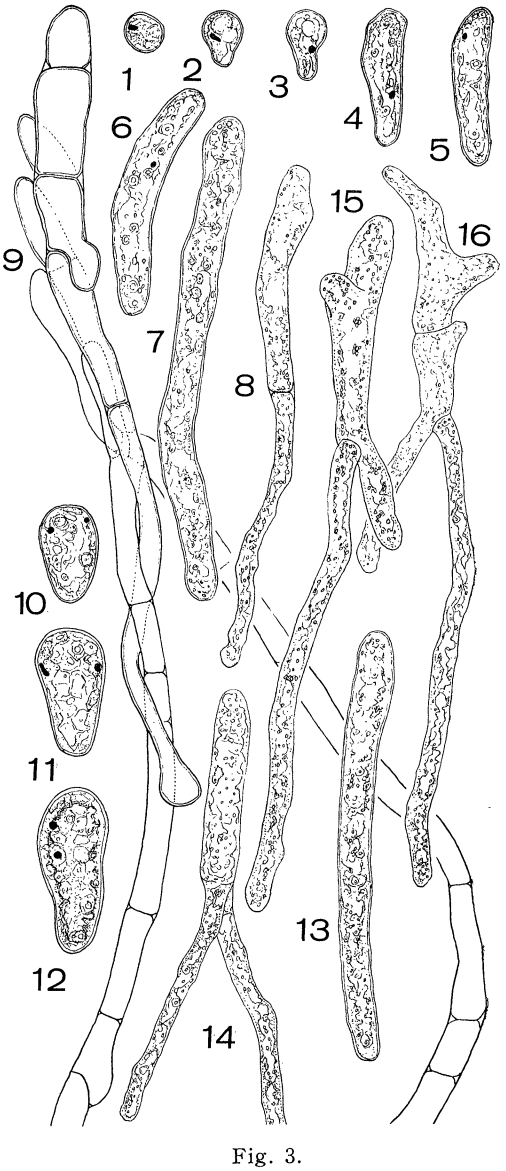

Fig. 3.

and Fig. 5-8, 9). The parthenogamete is also capable of germinating in the same method as the zygote (Fig. 3-13). This newly elongated portion is an initial cell which grows to become an erect system, which ultimately gives rise to a foliose and fan-shaped thallus by successive cell divisions. Sometimes a germling developes into an irregular expanded body (Fig. 5-12, 13).

After the germlings attain a considerable length, the first cell division usually occurs in transverse plane at the median part of the siphonous body. Then the upper cell produces a protuberance at its lower end. This protuberance elongates towards the direction of light and becomes a secondary rhizoid, whose shape is the same as the primary rhizoid (Fig. 3-14, 15, 16). The upper cell continues to grow, giving rise to a non-septate branch (Fig. 3-15, 16). As the branch grows longer, the septum is built at the base (Fig. 3-9, Fig. 4-2 and Fig. 5-10). This is a first lateral. Subsequent laterals are formed in the same way. As all laterals are built in this way and lie in one plane, a whole thallus becomes more or less fan-shaped (Fig. 4- 3, 5 and Fig. 5-11). Rhizoids repeat to produce branches which contribute to the for mation of a filamentous rhizoid system (Fig. 4-1, 2 and Fig. 5-10, 11). The germlings obtained in the present culture consist of both axial and intervening cell-filaments, and their general morphology, the method of lateral formation, the manner of ramification and the shape of chloroplast lead us to consider them to be a juvenile thallus of *Anadyomene wrightii*. However, they could not be cultured until their fertile adult stage.

7. Septum formation: The septum formation of both the upright system and the filamentous rhizoid system is not of the segregative type, but of the centripetal type as generally found in the members of the Cladophoraceae (Fig. 4-5).

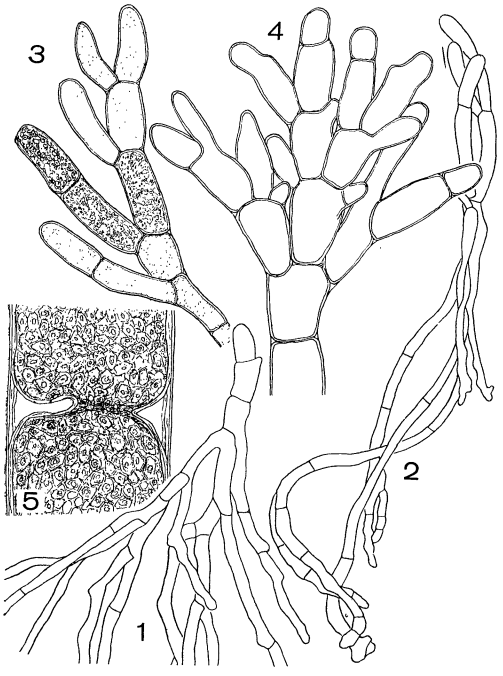

Fig. 4.

8. Cytological observation: Each component cell of the present alga is multinucleate, and several hundred neuclei are counted in each cell. The neuclei are globular and 3-5-8μ in diameter. A nucleus of resting stage contains a conspicuous neucleolus. Many small chromatin granules appear at the prophase when the neucleolus disappears. In prometaphase, chromatin granules are condensed to become well-defined chromosomes. Chromosome numbers can be counted in a polar view at metaphase, but an exact number of chromosomes can not be obtained. Twelve-fifteen chromosomes are counted in the germlings derived from zoospores (Fig. 6-5, 6, 7, 8). On the other hand, 25-30 chromosomes are counted in the germlings derived from zygotes (Fig. 6-1, 2, 3, 4). The number of chromosomes in the latter case has twice as many as that in the former one. Thus 12-15

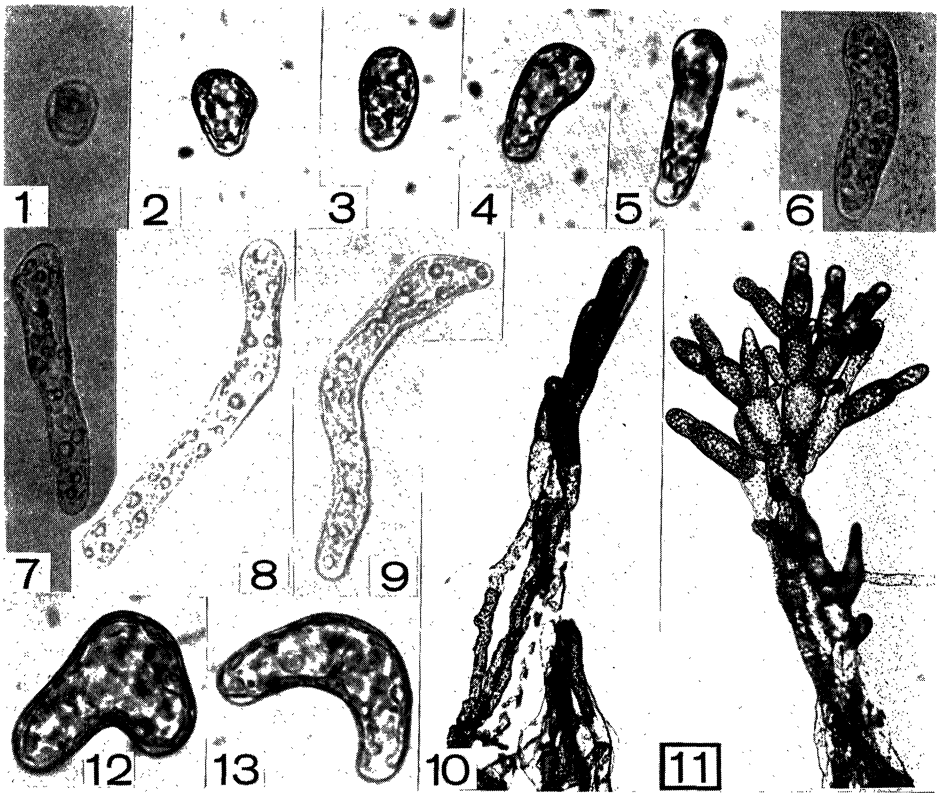

Fig. 5.

must be the haploid number and 25-30 must be the diploid number. As a nuclear membrane does not disappear during a nuclear division, showing so-called intranuclear mitosis, spindle fibers are not confirmed. The size of chromosomes are so small that we can identify neither the locations of the centromeres, details of chromosomal morphology, nor their karyotype. In the present study, we are not able to observe the meiotic division.

Discussion

The present culture study reveals that there are three types of plants in *Anadyomene wrigetii*; (1) plants producing zoospores, (2) plants producing male gametes and (3) plants producing female gametes. This result agrees essentially with those obtained by Iyengar and Ramanathan[7] and Jónsson[8] in their studies on the culture of *Anadyomene stellata*. We also confirmed that *Anadyomene wrightii* has an isomorphic alternation of generations and its gametophyte is heterothallis, as in the cases of *A. stellata*[7,8], *Microdictyon tenuius*[16] *Willeella japonica*[17] and many species of *Cladophora*[18].

When the well grown vegetative thalli are cultured in the laboratory, the thalli mature at 23°, but do not mature at 18°. This result agrees well with Jónsson's result[8]. He mentioned that the plant matured at 25°, but did not mature at 15°.

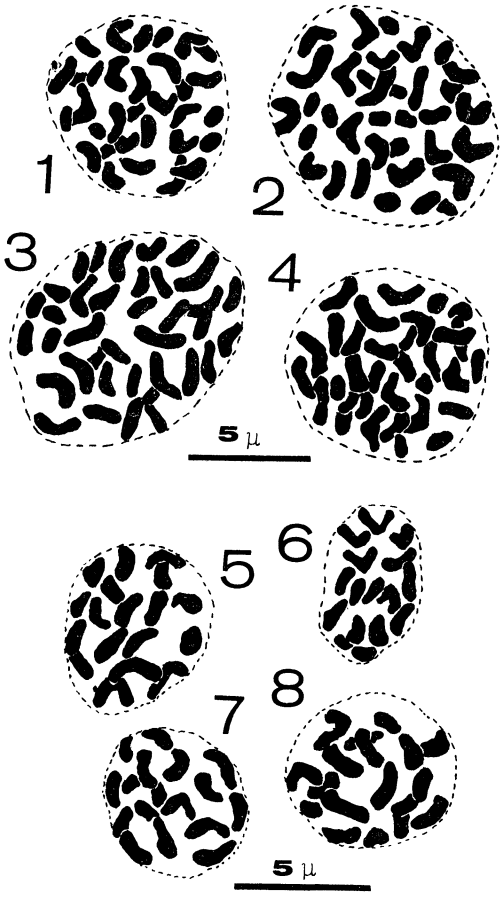

Fig. 6.

Judging from these results, we can suppose that sea-water temperature at 23° is indispensable to the maturity of *A. wrightii*. During our field observations on 25th to 28th May, 1967, matured thalli were encountered and the sea-water temperature was measured 23°-24°. As mentioned above, the swarmer liberation was encountered at 23° in the laboratory. This is almost identical with the water temperature in May-June (average 23.5°) at the collecting place of matured thalli.

We obtained both gametes and zoospores of the present alga at the same time in the same environmental condition. This evidence suggests that there is no seasonal difference in the alternation of generations of the present alga.

Our observations agree essentially with Jónsson's result[8] regarding the structure of the sporangium, the manner of swarmer formation, the shape of liberation pore, the mode of liberation and the manner of germination. However, the following minor disagreements were detcted: Jónsson observed that zoospores of *A. stellata* were 20.0-28.5× 10-15μ in size, possessing no papilla, but we observed that the zoospores of *A. wrightii* were a little smaller than Jónsson's, and they each had a conspicuous papilla.

Regarding the septal wall formation, no segregative formation was found in all parts of a thallus, but we observed only a centripetal formation. This type of formation has been recorded also on *Willeella japonica* by Chihara[17], on *Microdictyon japonicum* (Enomoto unpublished data), as in most members of Cladophorales.

Concerning cytological study of the present genus, there is only one report of *A. stellata* by Puiseux-Dao[19]. According to her descriptions, the resting nucleus had a neucleolus and numerous chromocenters, but she did not refer to the number of chromosome. In the present study, we observed that the germlings derived from zoospores have 12-15 chromosomes, and on the germlings derived from zygotes, 25-30 chromosomes were counted. Therefore, we assume that the reduction division takes place at the zoosporogenesis of the diploid plant.

Finally we wish to emphasize the taxonomic position of the Anadyomenaceae to which the genus *Anadyomene* belongs. The Anadyomenaceae has been arranged in the Cladophorales by Papenfuss[20], Dawson[21] and others. The present authors agree with the opinion of those scholars that *Anadyomene* as well as *Microdictyon* and *Willeella*, show closer affinity with the Cladophorales than with the members of the Siphonocladales, mainly basing on the presence of the centripetal formation

of septal walls and of the alternation of isomorphic generations, in which haploid gametophyte alternates with diploid sporophyte.

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Explanation of Text-figures

Fig. 1. Reproductive organs of *Anadyomene wrightii*.
1. Vegetative thallus. ×0.6.
2. Several sporangia with conical outgrowths. ×333.
3. Net-work of chloroplasts of sporangia before maturation. ×313.
4. Sporangia that are either empty or filled with swarmers. On the surface of empty sporangia are clearly seen liberation pores. ×160.
5. Swarmers in a sporangium. ×313.
6. and 7. Liberation of swarmers and liberation tube. 6. ×466, 7. ×666.
8. Circular openings of liberation pores. ×333.
9. Protruded liberation tubes and their openings. ×600.

Fig. 2. Zoospores, gametes and planozygotes of *Anadyomene wrightii*.
1. and 2. Zoospores. 1. ×1366, 2. ×666.
3. Zoospores stained with gentian violet. ×600.
4. and 5. Gametes. 4. ×1366, 5. ×666.
6. Gametes stained with gentian violet. ×600.
7, 8. and 9. Copulation of gametes. 7. ×733, 8. ×1366, 9. ×1366.
10. Planozygotes a little before complete fusion. ×1366.
11. and 12. Standstill planozygote just after deprived of flagella both. ×1366.
13. Planozygotes stained with gentian violet. ×600.

Fig. 3. *Anadyomene wrightii*. Developmental stages of zoospores, zygotes and parthenogametes.
1. Settled zoospore whose cell wall was already built, after 10 hours. ×625.
2. Beginning of the zoospore germination, after 1 day. ×625.
3. Germinating zoospore, after 5 days. ×625.

4. and 5. Clavate germling derived from zoospore whose cell content never migrates. 4. after 7 days ×475. 5. after 8 days ×475.
6. Germling derived from zoospore whose stigma still remains, after 13 days. ×475.
7. Siphonous, non-septate germling derived from zoospore, after 15 days. ×275.
8. Germling derived from zoospore that is undergoing first cell division perpendicular to the long axis of the germling, after 18 days. ×140.
9. Germling derived from zoospore, after 80 days. ×107.
10. Germinating zygote, after 3 days. ×850.
11. Germinating zygote, after 5 days. ×850.
12. Germling derived from zygote, with two stigmata which still remain after 7 days. ×850.
13. Siphonous, non-septate germling derived from parthenogamete, after 15 days. ×270.
14. Germling derived from zygote, with two rhizoids, after 25 days. ×130.
15. Germling derived from zygote which is issuing a first lateral, after 28 days. ×130.
16. Germling derived from zygote which is issuing laterals, after 30 days. ×130.

Fig. 4. Developmental stages of *Anadyomene wrightii*.
1. Germling derived from zoospore, with a bundle of rhizoids, after 130 days. ×35.
2. Germling derived from zygote, after 130 days. ×35.
3. Upper portion of germling derived from zygote, after 150 days. ×60.
4. Upper portion of germling derived from zoospore, with axes and intervening laterals, after 180 days. ×60.
5. Centripetal septum formation. ×280.

Fig. 5. Microphotographs of *Anadyomene wrightii*.
1–9. Germlings derived from zoospores. all ×833. 1. after 1 day, 2. after 2 days, 3. after 4 days, 4. after 5 days, 5. after 8 days, 6. after 9 days, 7. after 10 days, 8. after 12 days and 9. after 14 days.
10. Germling derived from zoospore, after 130 days. ×66.
11. Germling derived from zoospore, after 180 days. ×90.
12. and 13. Irregularly expanded germlings derived from zoospores, after 10 days. both ×666.

Fig. 6. Chromosomes of *Anadyomene wrightii*.
1–4. Chromosomes of the germling derived from the zygote. ×2533.
5–8. Chromosomes of the germling derived from the zoospore. ×2800.