On Some Culture Experiments with the Swarmers of Certain Species Belonging to the Ulvaceae

By

YUKIO YAMADA and EIJI SAITO

With Plate XVI.

On the life history of the Ulvaceae there have hitherto been published several reports by many investigators. SCHILLER (1907) studied the development of *Ulva Lactuca* and reported that there are three kinds of gametes, that is to say the macrogamete, the parthenogamete, and the microgamete. FOEYN (1929), working on the same species, reported that there is an alternation of morphologically similar generations and strictly separated sexuality in *Ulva*. He also studied its cytology and found that the sexual plant has 13 chromosomes, while the asexual individual has 26 chromosomes. MIYAKE and KUNIEDA (1931), working on a certain *Ulva* species which grows in the vicinity of Misaki, reported that they had obtained 2-ciliated zoospores. Also in *Enteromorpha* an alternation of morphologically similar generations was reported by HARTMANN (1929), KYLIN (1930), and MIYAKE and KUNIEDA (1931). According to BLIDING (1933), however, *Enteromorpha Linza* and *E. procera* possess only 4-ciliated asexual swarmspores. Judging from the reports, brought by REINKE (1878), CARTER (1926), and MIYAKE and KUNIEDA (1931), it had been expected that the zygote of *Monostroma* may germinate after a long resting period. KUNIEDA (1934) studied a certain *Monostroma* species, which grows in the vicinity of Misaki, and reported that the zygotes of *Monostroma* gradually increased in size until they attained maximum size after four to five months, then they became zoosporangia containing a number of 4-ciliated zoospores, altogether 32; after being liberated from zoosporangia the zoospores immediately germinated and developed into sexual plants. Because of this result he proposed to establish a new family, "Monostromaceae".

BLIDING (1935) found, however, in *M. Wittrockii* only 2-ciliated gamete-like swarmers, which immediately developed without conjugation.

The present writers have for several years carried on culture experiments with the swarmers of *Ulva pertusa* KJELLM., *Enteromorpha Linza*

(L.) J. AG., *Monostroma angicava* KJELLM., *M. pulchrum* FARLOW, etc. in the laboratory of the Institute of Algological Research at Muroran, and their results will be described in the following pages. The writers wish to acknowledge the assistance of Mr. T. KANDA, and also to express their thanks to the Hattori Hōkō Kai whose pecuniary assistance helped them greatly to carry on the present experiments.

Material and Method

All the materials used in the present investigation were collected at Muroran, near the laboratory of the Institute of Algological Research.

After having been brought to the laboratory the material was rinsed carefully with clean filtered sea-water, and each plant was placed in different glass vessels containing filtered sea-water. When swarmers had been discharged, a drop of water containing them was taken from one vessel on a slide-glass, and it was mixed with another drop from different vessel, and all such possible combinations among them were made under the microscope.

As a result of the examination it was found that there are two kinds of gametes in *M. angicava* and *Ulva pertusa*, while only zoospores are found in *M. pulchrum*, and *Enteromorpha Linza*. The proper quantities of swarmers were poured into glass vessels, containing filtered sea-water in order to fasten swarmers to slide-glasses which had previously been set against the wall of the vessels. When swarmers had come to rest the slide-glasses were transferred to the culture vessels. Observations were made under the microscope using these slide-glasses.

As the culture solution natural sea-water, filtered through a layer of absorbent cotton was used. It was renewed two or three times every month.

The culture vessels were placed in a room with north exposure where they were never exposed to direct sunlight.

The cilia of swarmers were stained with Rosanilin or Gentian-violet.

Observations

(I) Ulva pertusa KJELLM.

From January to October *Ulva pertusa* is found very commonly in the vicinity of the laboratory. The formation of swarmers first begins at the upper margin of the frond and extends gradually downwards. The fertile parts can easily be recognised by their yellowish-green or rather yellowish-brown colour. The swarmers escape through pores formed in

the membrane on both surfaces of the thallus (Text-fig. 1). Text-fig. 1, A is a surface-view of emptied fertile cells with pores, and B is a cross-section through the fertile part showing the liberation of swarmers.

As a result of the microscopical examination of 75 plants, the writers found that only 3 of them produced larger 4-cilated zoospores and the rest produced 2-ciliated gametes. It is impossible to distinguish morphologically the zoospore-bearing plants from the gamete-bearing ones.

SCHILLER (1907), FOEYN (1929), and MIYAKE and KUNIEDA (1931) reported that the swarmers of *Ulva* are liberated in the early morning, but according to the present writers' observations, the fully matured fronds liberated swarmers at any time of the day. They gathered densely on the lightest face of the vessel forming a green streak.

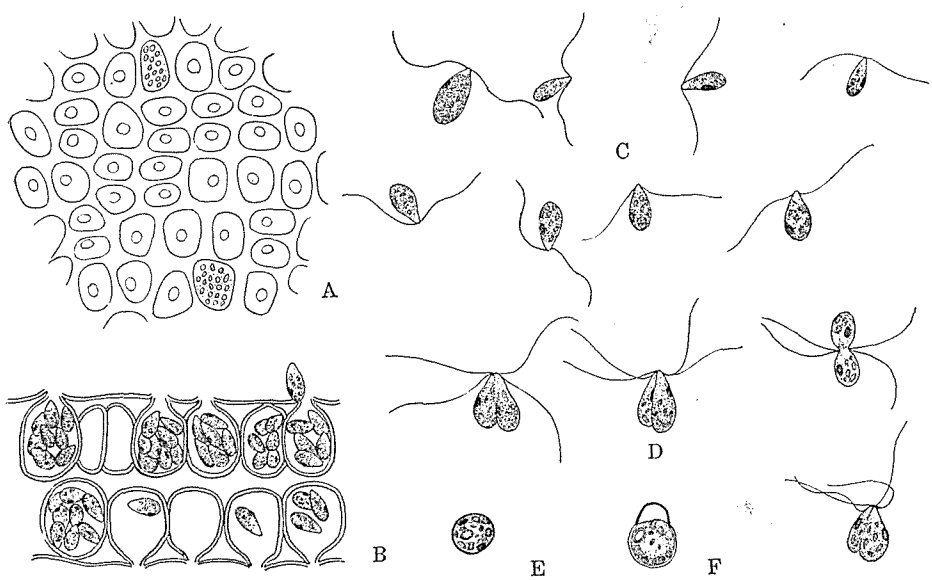

Fig. 1. *Ulva pertusa* KJELLM. A. Surface-view of the emptied fertile cells with openings. B. Cross-section through the fertile part. C. Gametes. D. Conjugation of gametes. E. Zygote. F. Germination of zygote. A–B. ×480, C–F. ×950.

The gametes are elongated pear-shaped, about 5.1–7.7 $\mu \times$ 2.0–3.4 μ in size, and they have two cilia at the anterior end and one chloroplast with an eye-spot in the posterior part (Text-fig. 1, C). The zoospores which are also elongated pear-shaped, about 10.2–11 $\mu \times$ 4.25–5.1 μ, have four cilia at the anterior end and one chloroplast with an eye-spot in the posterior part (Text-fig. 4, A). The length of the cilia of zoospores is about one and a

half times as long as the body itself, while it attains about twice the body length in gametes. MIYAKE and KUNIEDA (1931) reported gametes with a cilium-like appendage at the posterior end, which could not be found by the present writers.

1. Conjugation of gamates and development of zygotes

MIYAKE and KUNIEDA (1931) examined several hundreds of plants of *Ulva* and reported that only 8 plants of them gave gametes. On the contrary in the present investigation 72 gamete-bearing plants were found while only 3 discharged zoospores. As a result of all possible combinations of gametes a sharply separated sexuality was recognised as mentioned by FOEYN (1929) in *Ulva Lectuca*. A relative sexuality like this had already been mentioned by HARTMANN (1929) in *Enteromorpha ramulosa*. The gametes show considerable variation in size, but no difference in size was found in connection with their sexes.

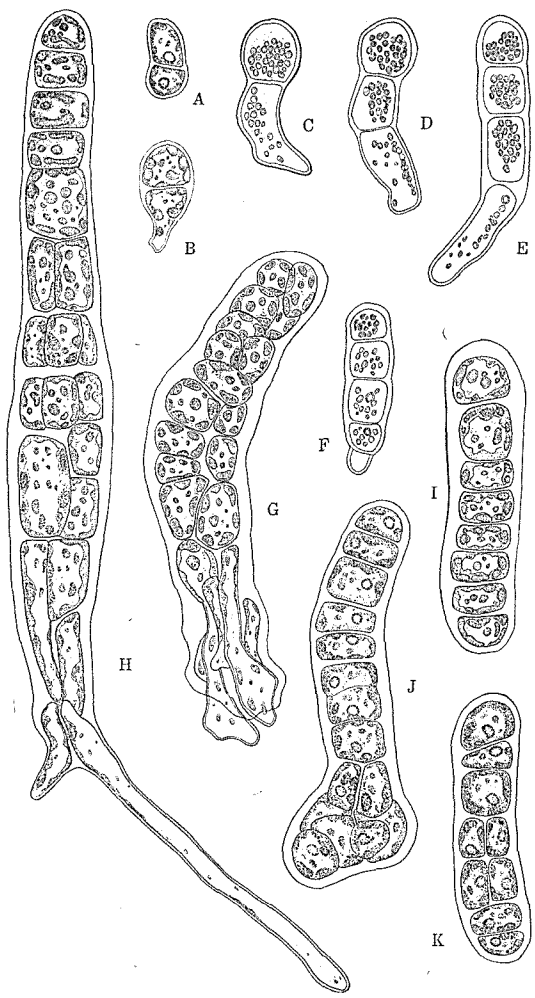

Fig. 2. *Ulva pertusa* KJELLM. A–C. Divided zygotes. D–H. Further development of zygote. G–H. 30-days old. I–K. Young plants without rhizoid. ×650.

The gametes conjugate generally side by side, but rarely attach end to end at the anterior ends to form zygotes. (Text-fig. 1, D). The zygotes swim about in a lively manner for a while with four cilia, but gradually become less active, and sooner or later cease to move and enter into a resting

stage. The 2-day old zygotes become spherical, about 6 μ in diameter, in which two eye-spots and one pyrenoid are still to be recognised. (Text-fig. 1, E). The 4-day old zygotes are invested with a clear membrane and begin to germinate; at this stage the eye-spots can not be seen. (Text-fig. 1, F). After about ten days zygotes divide into two to four cells, one of which elongates and forms the primary rhizoid (Text-fig. 2, A–E). By further cell-divisions and elongation young sporelings develop usually into a simple filament, consisting of a single row of cells. After about one month the cell-divisions with longitudinal walls begin to take place and secondary rhizoids are formed (Text-fig. 2, G–H). Sporelings are also often seen consisting of cells of nearly equal size and without the primary rhizoid (Text-fig. 2, I–K).

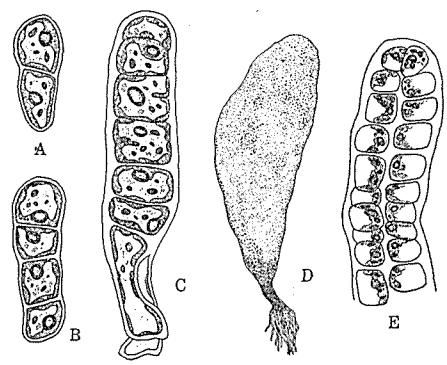

Fig. 3. *Ulva pertusa* KJELLM. Parthenogenetic development of gamete. A. 20-days old. C. 50-days old. D. 4-months old. E. Cross-secton of the same. A–C. × 650, E–F. × 325.

The parthenogenetic development of some gametes was also observed (Text-fig. 3, A–E).

2. Development of zoospores

The zoospores are less active than the gametes in respect to motion. After swimming about for a while the zoospores lose their cilia and come to rest. Within two or three days resting zoospores begin to germinate by pushing out a protuberance (Text-fig. 4, B–F). At this stage a pyrenoid is distinctly visible at the posterior end. After about 10 days the cell divides into two, one of which elongates and forms a primary rhizoid (Text-fig. 4, G). By successive cell-divisions with transverse walls there is formed a simple filamentous body with an elongated rhizoid cell (Text-fig. 4, H–K). The cell-divisions with longitudinal walls take place after about a month (Text-fig. 4, N–M). Young branched or bending sporelings were also found consisting of cells of nearly equal size, without a primary rhizoid (Text-fig. 5, A).

After about two months the sporelings developed into membraneous structures, which represent the young Ulva plants (Text-fig. 5, B).

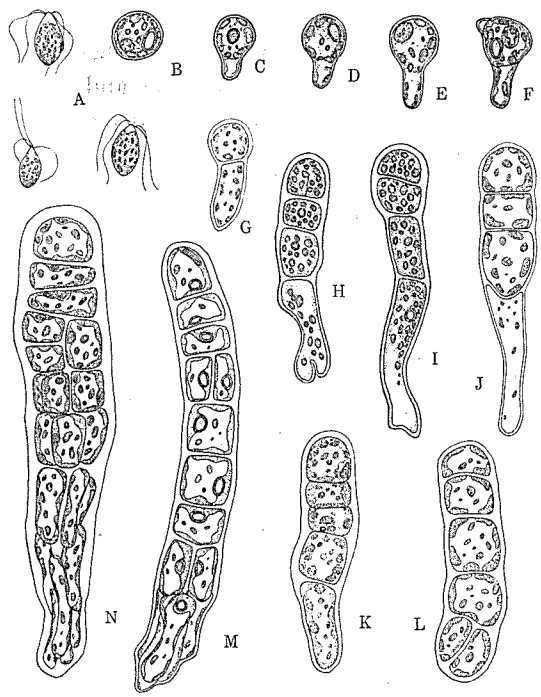

Fig. 4. *Ulva pertusa* KJELLM. A. Zoospores. B. Resting zoospore. C–F. Germination of zoospore. G–N. Further development of sporeling. H–J. 20-days old. M–N. 35-days old. ×650.

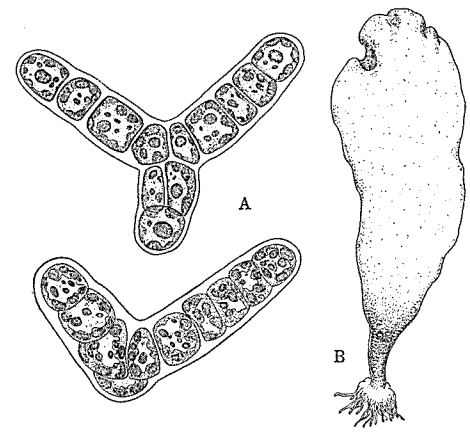

Fig. 5. *Ulva pertusa* KJELLM. A. Branched sporelings. ×650. B. 2-month old sporeling. ×10.

(II) **Enteromorpha Linza** J. AG.

In *Enteromorpha Linza* the fertile part of the frond is easily recognisable by its yellowish-green colour as in the case of *Ulva pertusa*.

In August and September the writers collected fertile fronds which were brought to the laboratory, and observations were made.

Each reproductive cell generally produces 8–16 swarmers (Text-fig. 6, A). The fully matured plants were carefully rinsed with clean sea-water, and placed in filtered sea-water, each plant in a separate glass vessel. Soon after being placed in vessels, all plants discharge a large number of swarmers forming a green streak from the yellowish-green upper margin of the frond. In 1936 examination was made of 151 plants of which 140 produced 4-ciliated zoospores, and only 11 plants 2-ciliated swarmers.

HARTMANN (1929) in *E. ramulosa* and *E. compressa*, KYLIN (1930)

in *E. intestinalis,* and MIYAKE and KUNIEDA (1931) in *Enteromorpha* sp. reported that the 4-ciliated zoospores are considerably larger than the 2-ciliated gametes, but in *E. Linza* only a slight difference in size between the 4-ciliated zoospores and the 2-ciliated swarmers was recognized. All possible combinations of the swarmers were made for letting zygotes form, but no success was achieved in observing conjugation among 2-ciliated swarmers. Of course no conjugation took place among zoospores. Among 2-ciliated swarmers produced from one and the same individual there were occasional 4-ciliated or 3-ciliated swarmers, on the other hand among the 4-ciliated zoospores from one and the same plant 2- or 3-ciliated ones were rarely found, and so the 2-ciliated swarmers may represent an abnormal form of the 4-ciliated zoospores. Moreover BLIDING (1933) stated that *E. Linza* possesses only asexual swarmers (4-ciliated zoospore-like neutral ones), so it is not probable that these 2-ciliated swarmers are gametes.

The 4-ciliated zoospores are elongated pear-shaped, measuring about 7.4–9.8 $\mu \times 4$–5.7 μ (on an average 8.3 $\times$ 5 μ), provided with 4 cilia at the anterior end, and one large chloroplast, an eye-spot, and one pyrenoid in the posterior part (Text-fig. 6, B). The 2-ciliated swarmers are also elongated pear-shaped, about 5.8–8 $\mu \times$ 3.5–5.2 μ (on an average 7 $\times$ 4.3 μ) in size, provided with 2 cilia at the anterior end, and one large chloroplast, an eye-spot and a pyrenoid in the posterior part (Text-fig. 8, A). The sensitiveness of both 4-ciliated zoospores and 2-ciliated swarmers to light seems weaker than that of *Ulva* as mentioned by KYLIN (1930) in *E. intestinalis.*

Ceasing their motion within two or three hours after being discharged both 4-ciliated zoospores and 2-

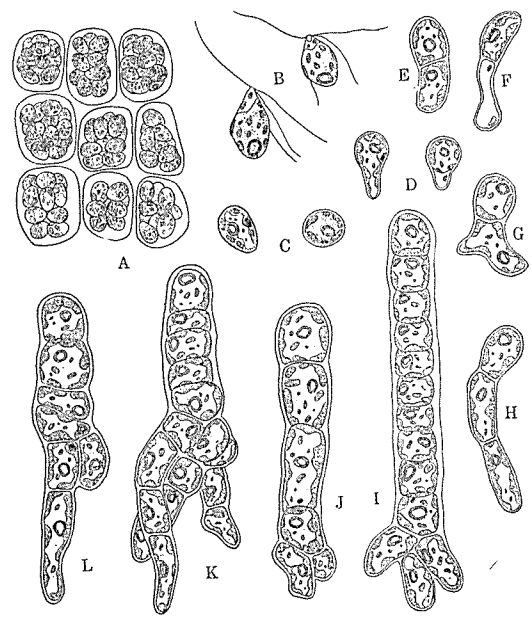

Fig. 6. *Enteromorpha Linza* J. AG. A. Surface-view of fertile part. B. Zoospores. C. Resting zoospores. D. Germination of zoospores. E–L. Further development of sporeling. A, C–L. $\times$ 650. B. $\times$ 1000.

ciliated swarmers come to rest. The resting swarmers are about 7 μ in diameter.

Development of swarmers

In two or three days both 4-ciliated zoospores and 2-ciliated swarmers begin to germinate by pushing out a protuberance (Text-fig. 6, C–D, Text-fig. 8, C–E). The eye-spot is distinctly visible for a few days. Within 2 or 3 days after that the cell divides into two or three. (Text-fig. 6, E–H, Text-fig. 8, G–K). By further cell-divisions each sporeling becomes a simple filament, consisting of a single row of cells with rhizoid cells, and in each cell a pyrenoid is clearly to be seen (Text-fig. 6, I–L, Text-fig. 8, L). After about a month the cell-divisions with longitudinal walls begin to take place, and the rhizoids considerably differentiate. Text-fig. 7 shows a 32-day old sporeling with well differentiated rhizoids.

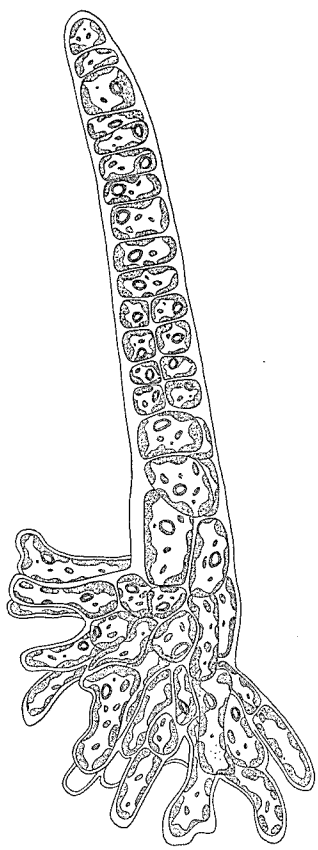

Fig. 7.
Enteromorpha Linza J. AG.
32-day old sporeling. × 650.

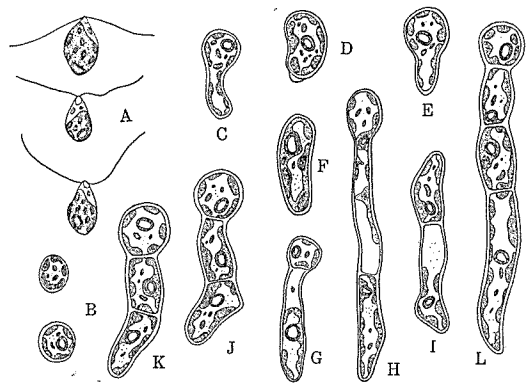

Fig. 8. *Enteromorpha Linza* J. AG.
A. 2-ciliated swarmers. B. Resting stage of the same. C–F. Germination of the same. G–L. Successive stages of development.
A. × 100. B–L. × 650.

(III) **Monostroma angicava** KJELLM.

M. angicava grows very commonly from January to May in the vicinity of our laboratory at Muroran.

Usually in March many fertile fronds of this alga which turned yellowish colour at the upper margin, are to be met with. In the experiments of 1936 fifty plants were rinsed carefully with clean sea-water, and placed in filtered sea-water, each plant in a separate glass vessel. Soon after being placed in vessels, 34 plants discharged numerous motile spores as a green streak from the yellowish-green upper margin of the frond. Each streak gathered densely at the lightest part of the vessel. It was ascertained that all plants produced 2-ciliated gametes and they escaped through the openings found always on the same side of the thallus as CARTER (1926) had reported. In surface-view these openings do not form such distinct round pores as in the case of *Ulva,* but only rents with very irregular margin. (Text-fig. 9, A). Text-fig. 9, B represents a cross-section through the fertile part of the frond, showing the liberation of the gametes.

There are two kinds of plants, the one is yellowish-green in colour in the fertile part of the frond, while the other is yellowish-brown, and the former produces larger gametes than the latter.

By trying all possible combinations of the gametes, it was found that only the gametes of different sexes could unite, while no conjugation took place among the gametes of the same sex. This sharply separated sexuality in *Monostroma* had already been proved by CARTER (1926), MIYAKE and KUNIEDA (1931), and by one of the writers, YAMADA (1932).

1. Gametes and their conjugation

There are two kinds of gametes, male and female, in *M. angicava.* The male gametes average smaller than the female in size. CARTER (1926) reported some difference of size in the gametes in connection with sex in *M. latissimum*; and the senior writer (1932) also in *M. angicava.* The male gametes are elongated pear-shaped, pale green, about $5–6\,\mu \times 2–3.5\,\mu$, while the female ones are also pear-shaped, but more rounded than the male ones, about $6–8\,\mu \times 4–5.5\,\mu$. The structure of the two kinds of gametes is very similar. They have two cilia of equal length at the pointed anterior end, and one chloroplast and an eye-spot in the posterior part (Text-fig. 9, C–D).

The gametes make very vivid movement vibrating their cilia, and remain motile for 2 or 3 days; they become rounded when the motion ceases.

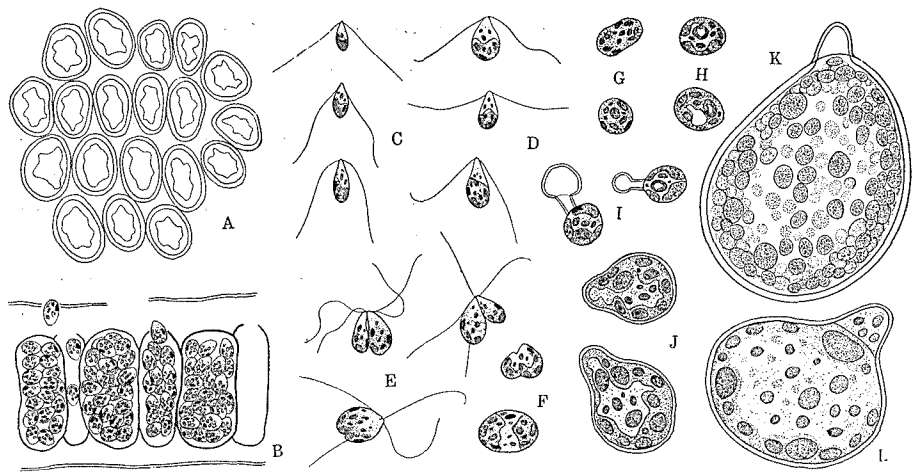

Fig. 9. *Monostroma angicava* KJELLM. A. Surface-view of emptied fertile cells with opening. B. Cross-secton of fertile part. C. Male gametes. D. Female gametes. E. Conjugation of gamates. F. Zygotes. G–L. Further growth of zygotes. G. 3-day old zygotes. H. 7-day old one. J. 16-day old one. K. 27-day old one. A–B, G–L. ×650. C–F. ×850.

The writers were not able to observe a gamete with a cilium-like appendage at the pointed posterior end, which has been reported by MIYAKE and KUNIEDA (1931).

It is very easy to recognize the conjugation of the gametes under the microscope by mixing both kinds of gametes on a slide-glass. Two gametes attach side by side, and swim about so rapidly and irregularly with 4 cilia as to be easily distinguished from gametes. They show negative phototaxis, and as their motion becomes less active, they gradually fuse together and finally round up when the motion ceases (Text-fig. 9, E–F).

2. Zygotes and their development

After stopping the motion, zygotes lose their cilia and come to rest being surrounded by a membrane. At this stage two chloroplasts and two eye-spots are to be identified (Text-fig. 9, F). The two eye-spots were distinctly visible for a few days, but after a week only one eye-spot was recognised in a few zygotes. As zygotes increase in size, their wall gradually thickens and the contents become densely green and full of starch (Text-fig. 9, J–L). After about three months the zygotes attain their maximum size, about 55–80 μ in diameter (Text-fig. 10, A; Pl. 16, 1). They

are spherical or somewhat elliptical in shape. (Usually they do not germinate, but in some cultures it was found that some young zygotes sent out a germination tube, the posterior end of which swelled and received the contents of zygotes. Then this swollen part increased in size (Text-fig. 9, I). Thence for about five months no change appears to take place either in size or in contents. Usually in the beginning of November zygotes become somewhat yellowish-green and are divided into many spores, each of which is provided with an eye-spot (Text-fig. 10, B; Pl. 16, 4). But their liberation is usually postponed until the end of November. (For example

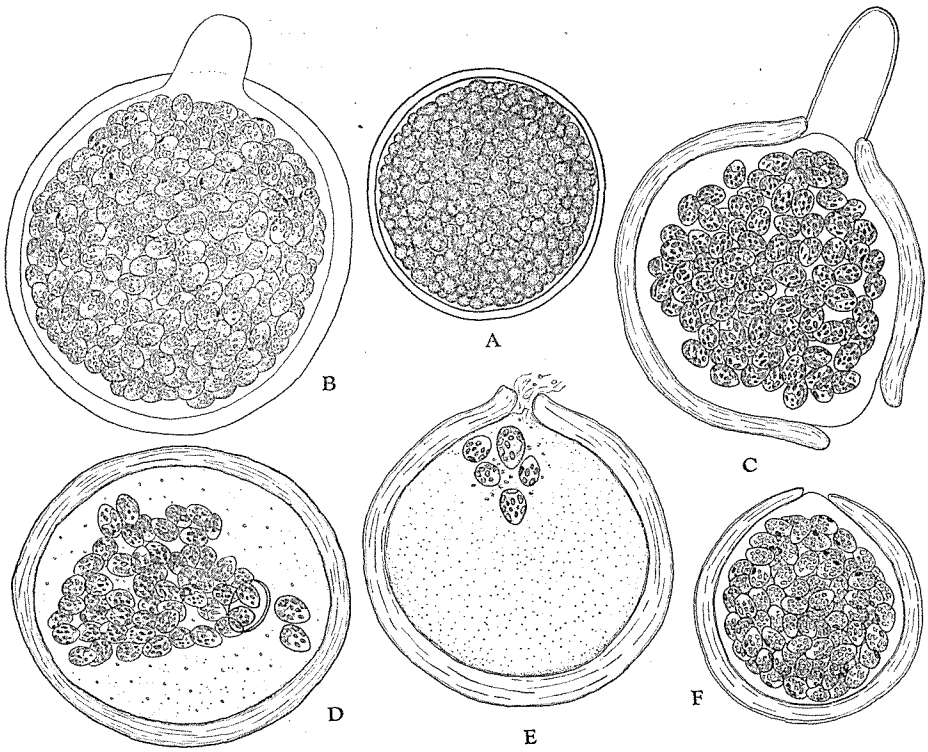

Fig. 10. *Monostroma angicava* KJELLM. A. 45-day old zygote. B–F. Zoosporangia. × 650.

the culture of zygote in 1936 was started on March 11th; on Nov. 8th the contents of some zygotes were found divided into numerous spores, and their liberation took place at first on Nov. 29th). When zygotes are ready for liberation of spores, the thick wall breaks and a rent is formed through which the spores escape one by one (Text-fig. 10, D–E). The writers

often met with a full matured zygote with two openings (Text-fig. 10, C). But sometimes the spores are discharged through a slender tube coming out from the surface of the zygotes, which can elongate to about 1–3 times the diameter of the zygotes. Such tubes appeared especially in the cultures made in 1937. It was easily clarified by staining cilia with gentian-violet that these spores were 4-ciliated zoospores. The number of zoospores produced in each zygocyst was not constant, but seemed to depend mainly on the size of the cyst.

3. Zoospores and their development

While the 4-ciliated zoospores are actively moving, they are pear-shaped, and about 6.0–9.4 $\mu \times$ 6.0–8.5 μ in size. They have four cilia at the anterior end, and a large chloroplast and an eye-spot in the posterior part (Text-fig. 11, A). They move less actively than the gametes, and their sensitiveness to light is not determined.

After swimming about for a while the zoospores lose their cilia and come to rest (Text-fig. 11, B), then soon begin to germinate by pushing out a protuberance, and at this stage a pyrenoid is clearly seen at its

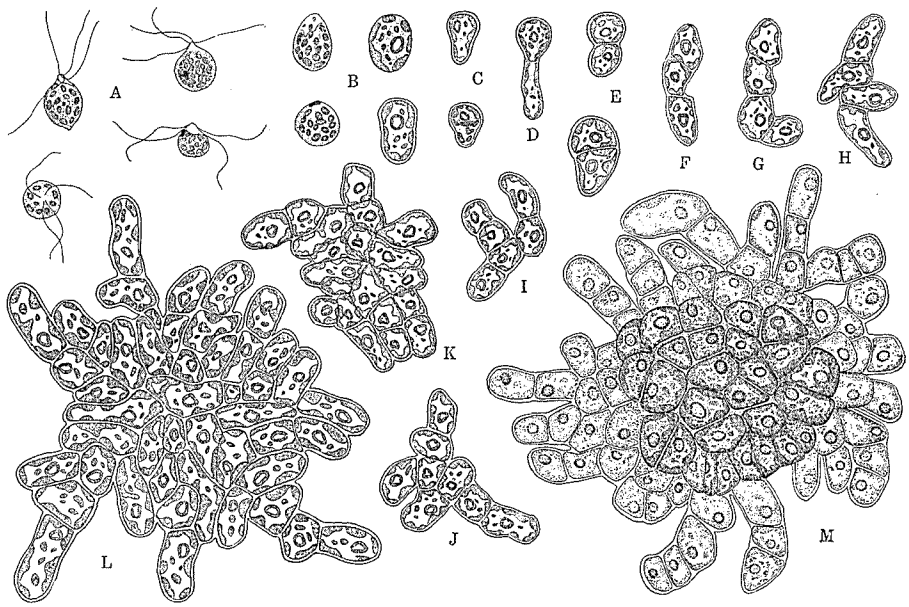

Fig. 11. *Monostroma angicava* KJELLM. A. Zoospores. B. Resting zoospores. C. Germination of zoospores. D–M. Further development of zoospores. A $\times$ 850. B–M. $\times$ 650.

posterior part (Text-fig. 11, C–D). Soon afterwards the cell divides with a transverse wall into two, and a pyrenoid is clearly seen in each cell (Text-fig. 11, E). When the cell divides into five or six, the sporeling begins to branch and fastens to the slide-glass (Text-fig. 11, H–K). By further development it becomes a disc and then begins to upheave in the central part (Text-fig. 11, L–M; Pl. 16, 3). Finally there is formed a standing sac-shaped swelling on the attaching disc. Though its further development beyond this stage has not been observed, it is probable that the germinating zoospores will produce a sexual plant.

It was often observed also that many zoospores had germinated in a zygocyst (zoosporangium) without swimming out from it.

(IV) **Monostroma pulchrum** Farlow

In the vicinity of Muroran *M. pulchrum* grows abundantly from January to June; it begins to reach the fertile stage from about the middle of February. When it comes to maturity, the marginal cells of the frond turn slightly yellowish white and seem just like small air-bladders. The fully matured fertile parts come off the frond and the marginal cells, each containing probably from 8 to 16 spores, fall to pieces, and then each cell wall breaks and swarmers are discharged. These swarmers vermiculate for a while attaching with their posterior ends to each other (Text-fig. 12, A).

The writers observed over a hundred plants and obtained only 4-ciliated swarmers from every plant.

Swarmers and their development

The swarmers are elongated pear-shaped or fusiform, measuring about 8–10 $\mu \times$ 3.5–4.5 μ; they have a number of granular substances, and are provided with four cilia, which are a little longer than the body itself, at the pointed anterior end, and one large chloroplast in the posterior part, but no eye-spot was observed (Text-fig. 12, B), and their sensitiveness to light was not recognised.

The swarmers move less actively than the gametes of *M. angicava*. After swimming about for 2 to 3 hours they round up and come to rest being surrounded by a membrane (Text-fig. 12, C). After resting for about five days, they push out a tube (Text-fig. 12, D), into which the cell contents begin to migrate. The distal end of the tube swells until it becomes much larger than the original spore, and almost all the contents of the original cell migrate into this enlarged part (Text-fig. 12, E). Thence

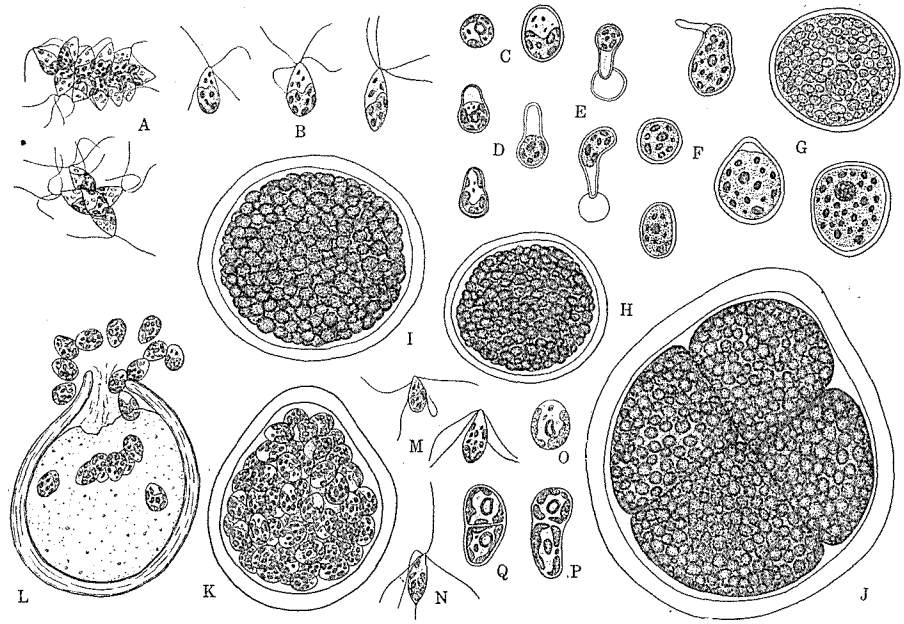

Fig. 12. *Monostroma pulchrum* FARLOW. A. 4-ciliated swarmers just discharged from mother-cells. B. Swarmers. C. Resting swarmers. D. Germination of the same. E. Migration of cell contents. F. 14-day old cells. G. 30-day old cyst. H. 3 month old cyst. I. 4 month old cyst. J. Cyst whose content is divided into four parts. K. Cyst containing zoospores. L. Liberation of zoospores. M–Q. Zoospores and their germination. B. ×900. A, C–Q. ×650.

the newly formed cell gradually increases in size, while the empty original cell begins to degenerate (Text-fig. 12, F); but it was observed that this protuberance elongated to about 25 μ in length in a few 14-day old materials. After 40 days almost all protuberances disappeared, and the cyst became a spherical or somewhat elliptical body, surrounded with a thick membrane, which is about 17–25 μ in diameter and full of starch (Text-fig. 12, G). Although after about 5 months the cysts attained maximum size, from about 50 μ to 80 μ, no change in its contents appeared to occur (Text-fig. 12, I). About eight months and a half after the cultures had been begun, however, it was recognized that a few cysts became somewhat yellowish green in colour and their contents were divided into from two to eight or more portions (Text-fig. 12, J). Then a few days later several cysts containing a large number of spores were met with (Text-fig. 12, K). Thus at the end of December the liberation of the swarmers could be observed under the microscope. When cysts arrived at the stage ready

for liberation of swarmers, the cyst walls suddenly broke and a pore was formed (Text-fig. 12, L). The swarmers are discharged through the pore in groups; they vermiculate for a while attaching by their posterior ends to each other, and then disentangle themselves from the group and swim off individually; they remain motile for 20 to 30 minutes. Contrary to expectation the swarmers were not 2-ciliated gametes, but 4-ciliated zoospores; of course no conjugation took place (Text-fig. 12, M). A few zoospores with a short cilium-like appendage at the pointed posterior end were very rarely met with (Text-fig. 12, N). The zoospores are elongated pear-shaped or fusiform, measuring about 6.0–8.8 $\mu \times$ 2.8–3.8 μ, contain a large chloroplast in the posterior part, but no eye-spot and their sensitiveness to light could not be recognised.

The writers failed to collect the zoospores on a slide-glass, which had been placed in each culture vessel, but some rounded zoospores and a few two-celled sporelings were found, in which the pyrenoid is distinctly visible (Text-fig. 12, O–Q). The further development of the sporelings was not observed.

As mentioned above it is to be noted that the 4-ciliated asexual swarmers are produced twice in the life-history of *M. pulchrum*.

Summary

I. *Ulva pertusa.*

It is impossible morphologically to distinguish the sexual plants from the asexual ones. The sexual plants are dioecious, showing a strictly separated sexuality. There is a considerable variation in size of the gametes, but no morphological difference according to sexes was observed; here occurs isogamy. The gametes are elongated pear-shape, having two cilia at the anterior end, and one chromatophore and one eye-spot in the posterior part. The zoospores are also elongated pear-shape, having four cilia at the anteiror end, and one chromatophore and one eye-spot in the posterior part. Both zoospores and gametes are positively phototactic. Both zoospores and gametes begin to germinate by pushing out a protuberance within two or three days after they have rounded. The cell divides with a transverse wall into two parts, one of which elongates and forms the primary rhizoid. By successive divisions with transverse walls the sporeling develops into a simple filament of cells, and then the cell-divisions with the longitudinal walls take place. The parthenogenetical development of some gametes was also observed.

II. *Enteromorpha Linza.*

There are two kinds of swarmers which are formed on different individuals, one having four cilia, but the other only two cilia instead of four. The number of individuals discharging swarmers with four cilia is much larger than the other. The form and the behavior of the swarmers are similar to those of *Ulva pertusa.* Within two or three days after they have been discharged, the swarmers begin to germinate by pushing out a protuberance. After four or five days the cell divides into two or three parts, one of which elongates and forms a primary rhizoid.

III. *Monostroma angicava.*

Only sexual plants were found. They are dioecious, showing a strictly separated sexuality. The male gametes are smaller in size and paler in colour than the female ones, and both are positively phototactic, while the zygotes show a negative reaction to light. After rounding up, the zygotes gradually increase in size and after about eight months they become zoosporangia which represent a very reduced asexual generation, and produce a large number of zoospores. The zoospores soon germinate and form small discs which afterward upheave at the central portion.

IV. *Monostroma pulchrum.*

All individuals which the writers have observed produced only swarmers with four cilia at the anterior end, and without an eye-spot. No sensitiveness of zoospores to light was observed. Within about five days after rounding up, the zoospores begin to germinate by pushing out a germination tube which becomes thickened at its end and receives the contents of the resting zoospore. Then this thickened part gradually increases its size, and after about eight months it produces a large number of swarmers. These swarmers are provided with four cilia at their anterior end, and have no eye-spot. They germinate asexually and some two-celled sporelings were observed, but their further development has not been followed.

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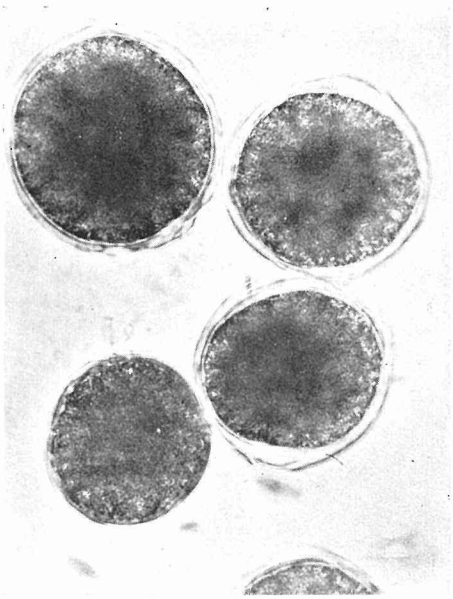
PLATE XVI

PLATE 16

1. *Monostroma angicava* KJELLM.
   Three-month old zygotes. ×400
2. *Monostroma pulchrum* FARLOW
   Three-month old zygotes. ×400
3. *Monostroma angicava* KJELLM.
   Disc-shaped sporelings. ×400
4. *Monostroma angicava* KJELLM.
   Matured zoosporangia. ×400



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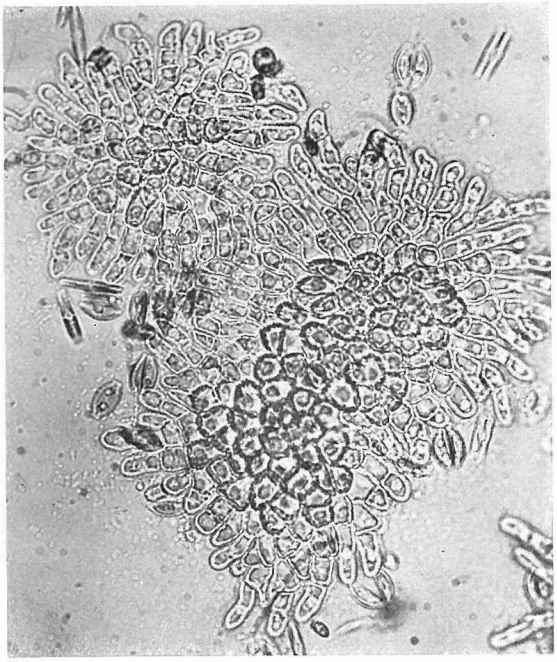

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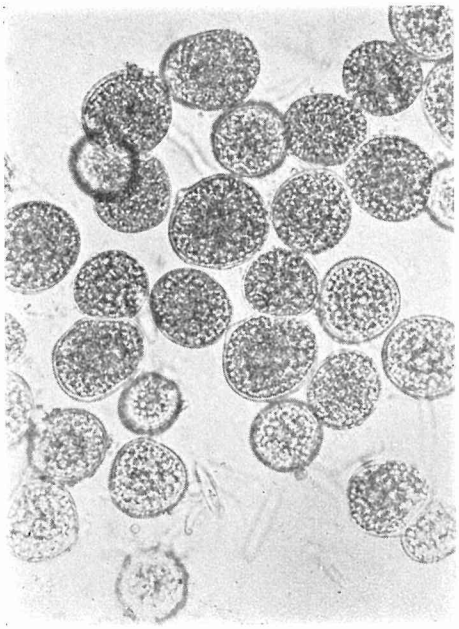

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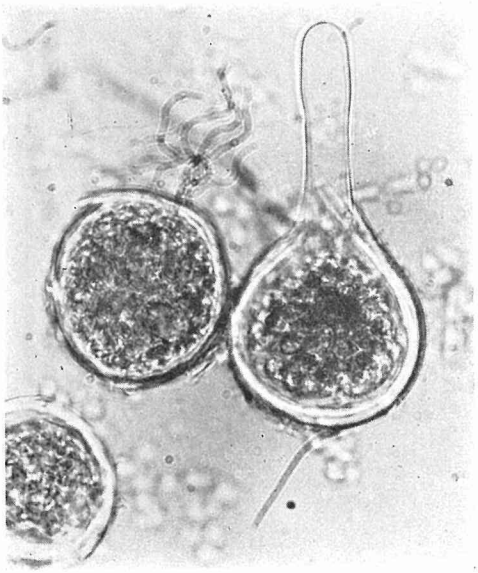

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