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Cytological Study of Swarmer Formation in *Enteromorpha Linza*

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Introduction

It is said that the identification of the members belonging to the genus *Enteromorpha* is very difficult, because they are so variable in form as the locality differs. The karyotype analysis is thought to be a good means to solve this problem, but such cytological studies on this genus are yet scanty.

The genus *Enteromorpha* is known to be a typical example of alga having iso-morphic alterations of generations, but a few strains which behave abnormally are also reported. These are well summarized by Tokida (1939).

Here, a member of this genus, *Enteromorpha Linza* (L.) J. Ag. is chosen as a material, and the cytomorphological observations will be reported. The main object of this work is to decide whether the first nuclear division in swarmer formation is meiotic or non-meiotic.

Materials and Methods

Enteromorpha Linza (L.) J. Ag. was collected at the Fukagawa seaside in Tokyo Bay, in November 1950 and in March 1951. Both samples do not differ morphologically each other. Fixation was made at an interval of one hour from 20:00 of the day of collection to 10:00 of the next morning. Navashin, Sanfelice, Flemming, Bouin-Allen's fluids were used as fixatives, and among them, Flemming and Bouin-Allen gave best results for both fixation and the following staining. Dehydration series started from 5 per cent ethanol, and chloroform was used as a solvent of paraffin. Sections were cut at 5 microns in most cases, and stained mainly with Heidenhein's iron haematoxylin. Feulgen's nuclear staining was also applied to the samples fixed with Flemming's fluid. The duration of hydrolysis with 1N HCl at 60°C was changed within 3–15 minutes, among which 5–7 minutes were best. Fixation of swarmers was made by osmic vapor, and stained with aceto-carmine. In other cases, carbol-fuchsin and haematoxylin were also used.

Observations

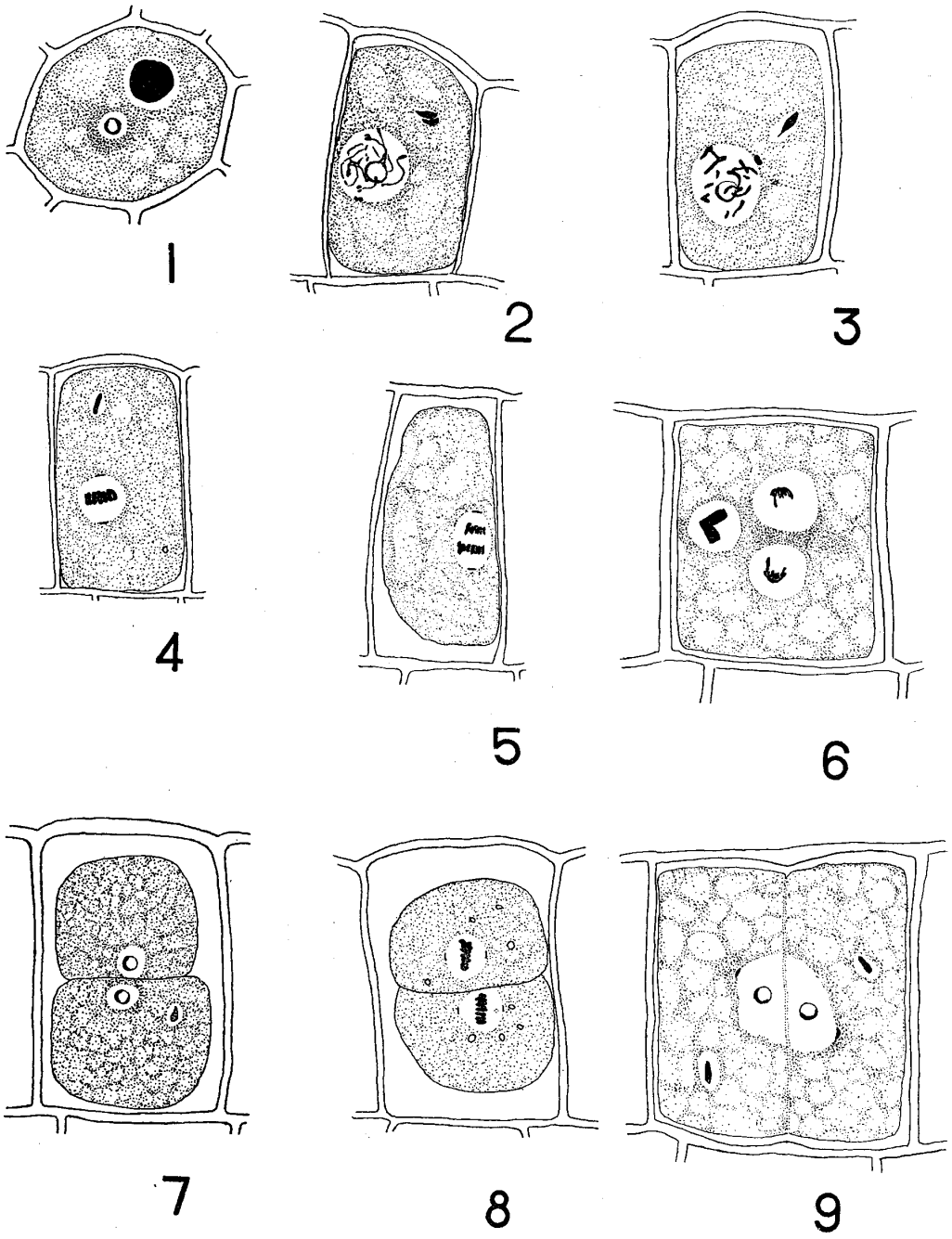
Resting stage—The outer half of the cell at the vegetative stage is very abundant in cytoplasm, and contains a chloroplast. In the residual half of the cell, the cytoplasm is less in quantity, and forms some vacuoles. A nucleus locates in the inner half, surrounded by a few mass of cytoplasm which stains more or less darkly. Chloroplast always contains a large, deeply stained pyrenoid. As the nuclear division draws near, the whole part of the cell becomes rich in the cytoplasm.

The resting nuclei are very small, 1.5–1.8 microns in diameter. The so-called net-work structure of the nucleus cannot be detected, and a rather compact, uniformly gray cavity is seen. In the center of this cavity, a nucleolus is situated. Almost all part of the nucleolus seems to be composed of a substance, having more transparent, and lower dense nature than that of the nuclear cavity, but a deeply stained region is always differentiated near the surface of the nucleolus.

Dividing stage—The axis of the first division in the swarmer formation is parallel to the surface of frond in *E. compressa* (Ramanathan, 1939), but is vertical in *E. Linza*. As the division begins, faintly stained thread-like structures appear in the nuclear cavity, and increase in number and stainability (Figs. 2, 12). The nucleus enlarges gradually, and the threads also become partially more thick, and finally take chromosomal appearance (Figs. 3, 13). Two or four of these chromosomes are always attached to the deeply stained region of the nucleolus. At metaphase, the nucleus decreases in its volume, and the shortened chromosomes are arranged on the equatorial plane. At this time, deeply stained structures, of which shape is creasant-like in the side view and cap-like in the polar view, appear at each pole of the spindle (Figs. 4, 13). These structures adhere very closely to the inner surface of the spindle, and thought to be centrosomes. Aster does not differentiated.

Since the chromosomes at metaphase are very small, the counting of their number is very difficult. The number is determined to be 12 (Fig. 10), but 10 in two instances. In the latter, however, there may be a miscounting, and the true number is probably 12, because four chromosomes lie very closely in two pairs. According to Ramanathan (1939), the chromosome number of *E. compressa* is 10 (n), and 20 ($2n$).

The nucleolus at metaphase decreases its volume, and the transparent part is almost disintegrated, while the deeply stained region remains adhering to the chromosomes. However, it is often observed that this region is separated from the chromosomes and exists inside of the spindle, rarely outside. And, if this region continues to connect to the arms of the daughter chromosomes, there occurs a bridge formation at anaphase. Bnt, in general, this deeply stained region of the nuc-



Figs. 1–8. Various stages of zoosporogenesis. Side-view, except Fig. 1. Fig. 1. Resting stage. Surface-view. Figs. 2–3. Prophase. Fig. 4. Metaphase. Fig. 5. Anaphase. Fig. 6. Telophase. Fig. 7. Interphase at 2-celled stage. Fig. 8. Anaphase at 2-celled stage.
Fig. 9. Telophase in vegetative cell division. All, ×1700.

leolus degenerated and disappeared into the spindle substance finally. When the groups of daughter chromosomes have reached to each pole, the spindle is furrowed and divided into two parts. These parts gradually decrease in volume, in which the chromosomes are getting out of shape at the center (Fig. 6). As the chromosomes lose their stainability, and disappear, the nucleolus is again constructed, and the resting daugh-

ter nuclei are formed (Fig. 7). The daughter cells, having been formed by a division vertical to the surface of the frond, divide next parallel to (Fig. 8). There is no sign of meiotic division not only in the first but also in the second division. Divisions continue further, and the swarmers are finally formed, but these processes could not be traced exactly.

Using the Feulgen's staining method, following results were obtained : the nuclear cavity at resting stage, centrosomes, nucleolus including a part stained deeply with basic days are all negative, while the chromosomes at metaphase and anaphase are clearly positive.

Fig. 10. Chromosomes at metaphase. ×3400

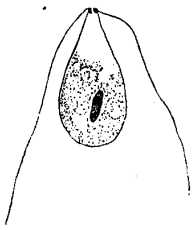


Fig. 11. Two-flagellated zoospore. Fixed with osmic vapor, and stained with aceto-carmine. ×1700

Swarmer cells—10 individuals were randomly picked up from the samples collected in November and March respectively, and in order to decide the nature of swarmers, all possible combinations between the groups of swarmers liberated from these individuals were examined. Conjugation did not occur in all cases, and the swarmers became rest after two hours from their liberation. From this result, it can be said that these swarmers have a nature of zoospore. Morphologically, there is no conspicuous difference between these and those reported by previous authors, excepting a fact that the number of flagellum is two (Fig. 11). Out of 80 individuals picked up from the samples of November and March, only one liberated four-flagellated zoospores, and all the remainings liberated two-flagellated zoospores. The cytology of this exceptional individual could not be done, and the observations described above are all in the materials having two-flagellated zoospores. The measurements of length and width of zoospores are as follows : 10.1 × 4.4 microns in four-flagellated, 9.5 × 3.8 microns in two-flagellated zoospores, but these scales are somewhat too large, because the zoospores are stained with aceto-carmine.

Considerations

It was very frequenty reported that the well-defined net-work structure of the nucleus as observed in higher plants, could not be detected in the chlorophyceous plants. Ramanathan (1939) also could not find such structure in the resting nucleus of *E. compressa*, and that accords well

with the result obtained in *E. Linza*. Regarding to the stainability of the nucleolus, however, there is a difference between these two members. In *E. compressa*, the nucleolus is stained uniformly with basic dyes, but in *E. Linza*, it has a differentiation. Namely, the nucleolus of *E. Linza* consists of a deeply stainable region and unstainable remaining part. The former part remains to later stage of the nuclear division, while the latter disintegrates at earlier stage, at which the chromosomes become more stainable.

Godward (1953), studying the relationships between the nucleolus and the chromosome in *Spirogyra*, states that the nucleolar substance first forms an outer covering of diffused material, which gradually condenses on the chromosomes, thus becoming 'chromosome substance'. Though the nucleolus of *E. compressa* seems to take no active part either in the formation of chromosomes or of the spindle (Ramanathan, 1939), the deeply stained region of the nucleolus observed in *E. Linza* may be functionally equal to that described as a nucleolar organizing truck by Godward (1950). This region is probably specialized part of the chromosome and/or nucleolus, through which the transference of substances is carried out at prophase.

Cap-like centrosomes were reported previously in *Ophiobolus graminis* (Jones, 1926). The mode of occurrence and behavior of the centrosomes are very differently reported by various authors and in various materials (cf. Yuasa, 1942). In the case of *E. Linza*, their origin may be intranuclear. In the nucleus of early prophase, there are often observed one or two granules different from the chromosomes in their apperance. If the stages are earlier, the number of granule observed is one, and later is two. They are found in the cavity of nucleus first (Fig. 12), and adhere to the inner side of nuclear surface at last (Fig. 13). The processes of degeneration and the relation to the structure of the swarmers are still obscure.

Fig. 12. Nuclei at early prophase.
Fig. 13. Nucleus just before metaphase. Note behaviors of granules, supposed as an origin of centrosomes. ×1700

Whether this strain of *E. Linza* is diploid or haploid is yet uncertain, though the occurrence of meiotic division cannot be observed in the course of swarmer formation. A strain, which was studied by Blidding (1933), has a life cycle only through the formation of zoospores, and is always diploid. Moewus (1938) states that, in three species including *E. Linza*, the diploidization of the chromosome number occurs during the development of haploid individuals which have been germinated from the gametes parthenogetically, and thereafter these diploid

individuals produce four-flagellated diploid zoospores. Yamada and Saito (1938), who observed the occurrence of both four- and two-flagellated zoospores in *E. Linza*, concluded that the latter was an abnormal type of the former. These previous investigations show that the strains carrying their life cycle only asexually is diploid in general. But comparing this strain with those reported previously as to the chromosome number and the scales of zoospores, the author is in opinion that the strain studied here is haploid and carries a life cycle only asexually. According to the genetically analysed data of Moewus (1938), the ability of parthenogenesis and the ability of chromosome diploidization are inherited both as monogenic. Accepting this fact, the following supposition becomes possible: one possibility is that the ability of parthenogenesis becomes strengthened to the extent that the conjugation is never necessary to carry the normal life cycle, and another is that the ability of chromosome diploidization is lost completely. If the combination of these two possibilities occurs in a strain, it may behave asexually through the course of its life cycle, and be always haploid. The exceptional strain producing four-flagellated zoospores, may be an original form from which this strain has derived, or may be a strain which originated from this strain as a result of chromosome diploidization. Beside these suppositions, the environmental conditions, such as local or seasonal, also be able to influence upon the expression of these characters.

Thus, the origin of this strain can be explained in several ways, but, of course, exact nature of this strain should be analysed by a further investigation on the artificial culture as well as in the field.

Summary

Swarmer formation in *Enteromorpha Linza* (L.) J. Ag., collected at the Fukagawa sea-side in Tokyo Bay, was investigated cytomorphologically. The number of chromosome was determined to be 12. The relations between the nucleolus and the chromosomes, and the behaviors of the cap-like centrosomes were described. The first and the second nuclear division in swarmer formation were both not meiotic. It was suggested that the strain used here had only haploid generation and propagated only by two-flagellated zoospore formation.

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