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A developmental mutation in *Enteromorpha lingulata* J. Ag. (Chlorophyta, Ulvales)

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A mutation which appears only in the haploid female state and affects the early development of *Enteromorpha lingulata* by disorienting direction of cell division is described. The atypical spheroid thallus is the result of interaction between Mendelian and non-Mendelian or extrachromosomal factors.

Introduction

Many cellular factors have been shown to affect morphogenesis in members of the Ulvales. Genetic phenomena in *Ulva mutabilis* Føyn have been examined by Føyn (1960, 1961, 1962), Løvlie (1964), Fjeld (1970) and Fjeld & Løvlie (1976). Several phenotypically spheroid to elongate mutants were examined and were found to possess Mendelian inheritance. In addition, Fjeld (1970) found a mutant (bu) which lacked complete control of its spindle orientation. The product of the wild type (bu^+) gene had a predetermined effect on offspring in early development by masking the mutation in a few successive generations. Gayral (1967) investigated a spontaneous mutant in another species, *U. fasciata* Del. No other multicellular marine green algae have been thoroughly studied genetically.

A variety of extracellular factors has also been shown to affect Ulvalean growth and morphology. Sunesson (1942, 1943) increased growth of *Enteromorpha* and *Ulva* with algal exudates and with sea water in which various algae had been immersed. Provasoli & Pintner (1964) showed that the morphology of axenic cultures of *Monostroma* and *Ulva* was affected by bacterial or red and brown algal exudates. The possible identity of these exudates and a review of the organic requirements of seaweeds, including vitamins and hormones, is given by Fries (1973). Because a large variety of compounds exists in natural sea water and affects seaweed morphology, it has been difficult to produce a synthetic medium capable of sustaining normal growth. Most recently, however, Bonneau (1977) found that axenic *Ulva lactuca* L. produced typical and atypical haploid thalli regardless of type of media or presence of microflora. Since these thalli occurred intermittently in successive lines, Bonneau postulated that 'non-mutational variability in phenotypic expression' was occurring.

After a clone of *Enteromorpha* had reproduced typical plants in our laboratory for about one year, a hollow, spherical thallus appeared among typical progeny (Fig. 1). When it was transferred to fresh medium, it produced plants which were intermediate between typical branched filaments and hollow spheres (Fig. 2). When these plants were transferred to fresh medium, a mixture of typical and spheroid plants was produced (Fig. 3). The purpose of this work is to determine whether or not the factors inducing the spheroid thalli have been described previously and whether they are cellular or extracellular in nature.

Materials and methods

The clone of *Enteromorpha* used in the study was isolated from the north jetty at South Padre Island, Texas, in September, 1974. It was identified as *E. lingulata* using the criteria of Kapraun (1969) for isolates from the Texas coast. This identification is tentative until further studies are done using southern Texas isolates. When the spheroid thallus appeared,

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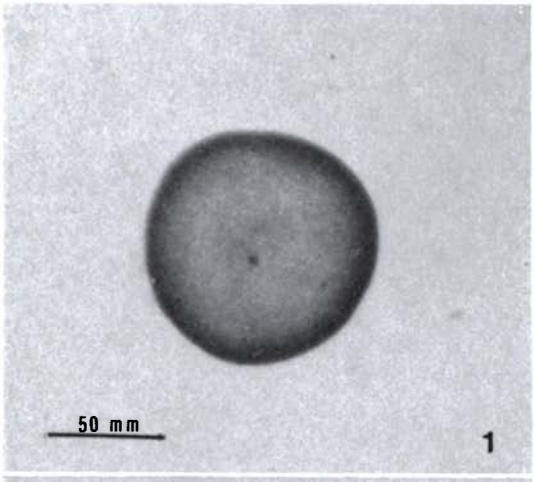

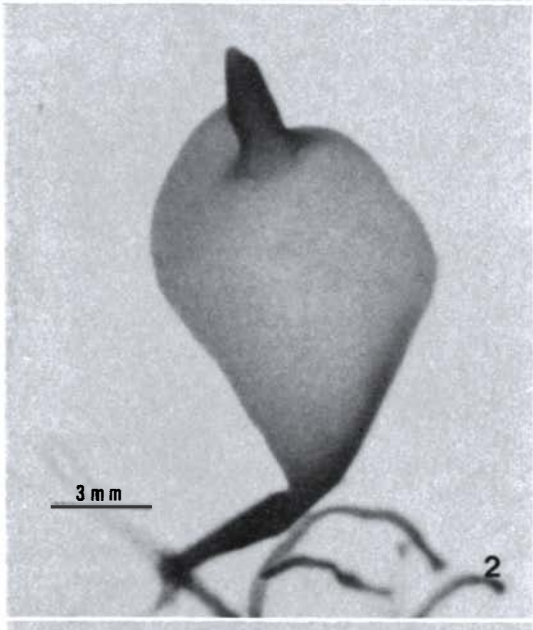

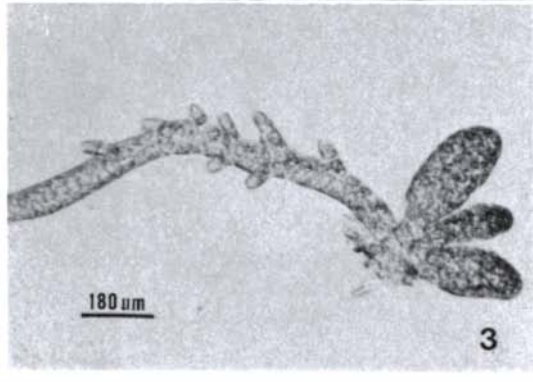

it and its progeny were individually transferred to fresh von Stosch enriched sea water medium (von Stosch, 1964). Representatives of morphologically distinct typical and atypical plants were transferred from all sequential generations and the lineages were recorded.

All cultures were maintained in an incubator at 20°C and at 16–$\overline{8}$ hr photoperoid. Light was provided by four Westinghouse 15 W cool-white fluorescent tubes with a surface illumination of 1·4 μW/cm^2-nm blue light and 0·65 μW/cm^2-nm red light, measured with a Plant Growth Photometer IL150 (International Light Co.). All experiments were done using unialgal rather than axenic cultures except for exudate and artificial media tests. Unialgal cultures were maintained in von Stosch enriched sea water medium using bay water at 35‰ salinity collected at South Padre Island. The sea water was stored in sealed plastic 19 l carboys in the dark where it was aged for a period of 21–90 days. Excessive ageing was avoided because it promoted anaerobic bacterial growth with a sulphurous odour. The medium was sterilized by heating to 72°C and cooling to 20°C for two consecutive periods which were 24 hr apart (double pasteurization or tyndallization). Unialgal cultures were maintained in 100 mm × 15 mm plastic disposable Petri dishes containing 25 ml medium, and were transferred weekly.

Axenic cultures of representatives of typical and spheroid morphologies were secured by transfers of 3–10-celled sporelings (by 'stabbing') into autoclaved 0·4% agarized von Stosch medium containing antibiotics. Antibiotics used were 1000 i.u. penicillin G plus 500 i.u. streptomycin S per ml (Fries, 1963) which were sterilized by millipore filtration. The treatment lasted 3–10 days and the thalli were frequently turned in the medium with sterile forceps. Each thallus was tested for purity by transferring a damaged portion to the surface of autoclaved von Stosch medium solidified with 1% potato dextrose agar and was checked microscopically for 2 weeks and throughout the experiments for presence of bacteria or fungi. If bacteria-free, the thallus was transferred to artificial media contained in 125 ml Erlenmeyer flasks. All glassware was cleaned by the

FIG. 1. The original spheroid thallus of *Enteromorpha lingulata* as it first appeared in culture.

FIG. 2. Diploid progeny of original spheroid thallus of *E. lingulata* which contained both filamentous and spheroid characteristics.

FIG. 3. Filamentous and spheroid sporelings produced in culture by the same sporophyte parent.

following series: distilled water rinse; 50% HNO_3 wash; distilled water rinse; 50% HNO_3 wash; three double distilled water rinses. Artificial media used for axenic cultures were KDX (Bonneau, 1977), $ASP_{12}NTA$ (recipe from L. Provasoli following Provasoli & Pintner, 1977) and Ott's artificial seawater medium (Ott, 1971); each was sterilized in the flasks by autoclaving.

Mating experiments between typical and spheroid gametophytes were performed according to some methods used on *Ulva mutabilis* by Fjeld (1970) and others. This involved inoculating large numbers of cultures with various ages and generations of plants to increase the chance of a male filamentous form and a female spheroid form releasing gametes at the same time. When this occurred, the gametes were placed in suspension on a glass slide which was half illuminated and half shaded. The gametes congregated in the lighted area, and upon fusion, quadriflagellate zygotes moved to the dark portion and settled. Single zygotes were transferred to fresh von Stosch medium and their development and progeny were followed for several successive generations.

To determine the effect of algal exudates on typical and atypical thalli, axenic (filter sterilized) preparations from one morphology were added to the axenic cultures containing the opposite morphology. The exudates were prepared either by freezing thalli of different ages and homogenizing them in a sintered glass homogenizer or by homogenizing live plants of various ages, from sporeling to senescing stages; the liquid was filter sterilized.

To determine if cell division rhythm differed between typical and atypical thalli and to find variations in ontogeny, the cells were treated in 0·1 M HCl at hourly intervals during the dark period. Nuclei were stained with aceto-carmine to find mitotic figures in a cell-squash type procedure.

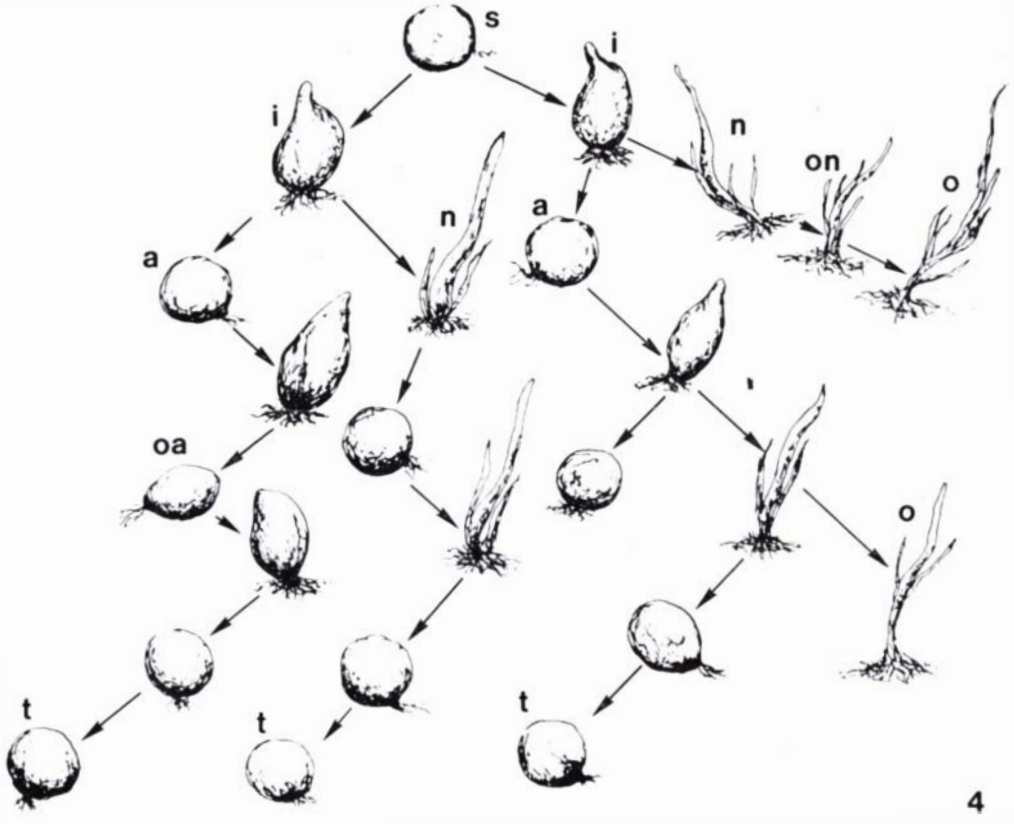

FIG. 4. Selected lines from transfer experiments of the *Enteromorpha* mutant: s = original spheroid thallus, i = intermediate sporophytes, a = atypical spheroid thalli, n = typical filamentous thalli, on = plants producing only typical forms, oa = plants producing only atypical spheroid forms, o = typical thalli producing only typical thalli, and t = atypical thalli only producing atypical spheroid thalli.

Results

A portion of the major lines of the transfer experiments is shown in Figs. 4. The original spheroid thallus (s) produced biflagellate swarmers which presumably underwent diploidization to produce intermediate forms (Figs 2 and 4i). These forms had typical basal portions and elongate apical portions, but were centrally spheroid. They were considered diploid because they produced quadriflagellate zoospores which, upon germination, gave rise to both atypical and typical thalli (Fig. 4 a, n, respectively).* All of the morphologically distinct typical and atypical forms selected for transfer produced biflagellate gametes (Fig. 5), and were thus considered haploid gametophytes. Some lines in the transfers produced only typical or only atypical forms (Fig. 4on, oa, respectively) but most produced mixtures for several generations. As shown in Fig. 4, some lines alternated between producing mostly typical and mostly atypical forms. At the conclusion of the transfer experiments and at present, typical thalli (o) only reproduce themselves by spores formed by diploidization or by either anisogamete. Atypical thalli (t) only reproduce themselves parthenogenetically by the larger anisogamete, and since only biflagellate gametes are produced, the atypical thalli are considered haploid. Diploidization no longer occurs in the atypical thallus, as evidenced by its lack of quadriflagellate spore production. Other atypical thalli were noted in the haploid progeny and are worth brief mention. One thallus arose in the haploid progeny of one spheroid thallus which produced rhizoidal holdfasts from several groups of cells scattered over the thallus (Fig. 6). Rhizoids are much reduced in all other atypical thalli and are only noticeable in sporelings (Fig. 7). One original sporophyte (Fig. 4i) produced an atypical spheroid thallus with uniseriate branches (Fig. 8). A third atypical thallus was noted which appeared among haploid progeny and had a development similar to that of *Monostroma*. As the spheroid thallus matured, it split apically and longitudinally to produce a flat, monostromatic thallus (Fig. 9). None of the three abnormalities have

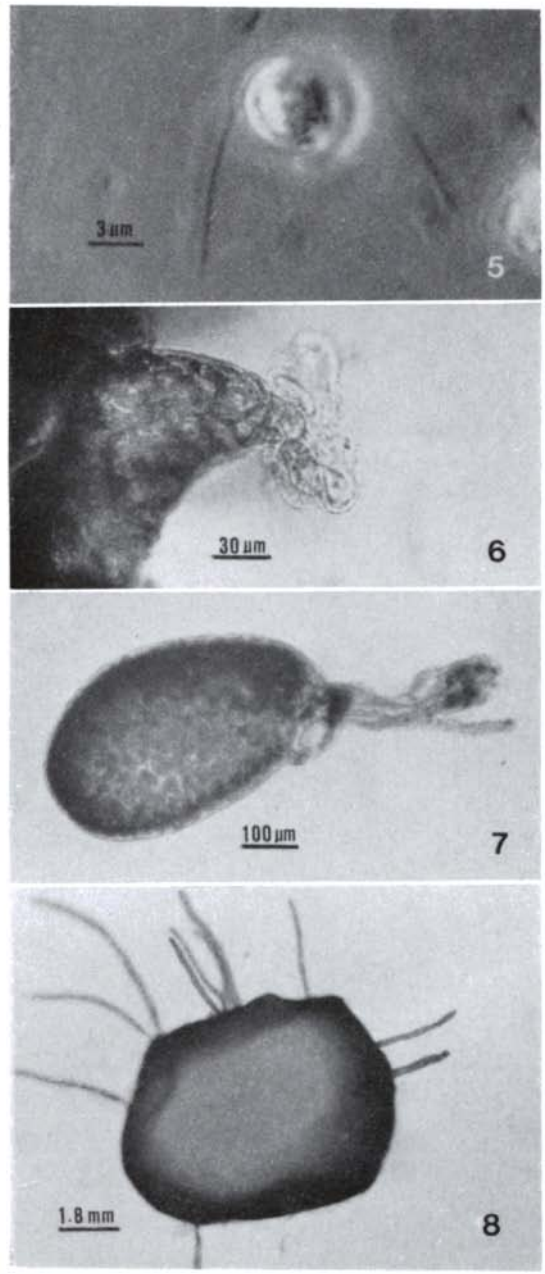

FIG. 5. Phase contrast micrograph of gamete of *Enteromorpha lingulata* after attachment to glass slide.
FIG. 6. Adventitious rhizoid formation on spheroid thallus.
FIG. 7. Spheroid sporeling showing basal rhizoids.
FIG. 8. Spheroid thallus with infrequently produced branches.

* Using flagellar number to indicate ploidy is not always valid. As an added indication of ploidy, it was noted that biflagellate swarmers were anisogamous, were positively phototactic and became negatively phototactic upon fusing. Quadriflagellate zoospores were rarely produced, but in one case a typical plant produced some which were observed to be negatively phototactic immediately prior to settling.

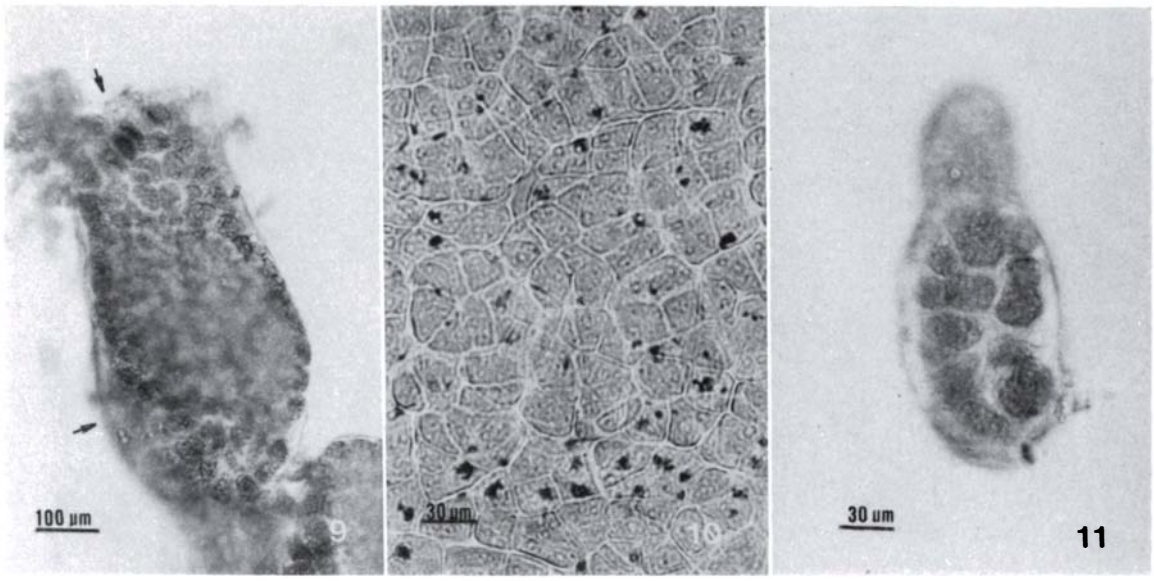

FIG. 9. Atypical spheroid thallus of *Enteromorpha lingulata* with apical and lateral ruptures simulating monostromatic condition.

FIG. 10. Acetocarmine stained preparation showing synchronized cell division in spheroid thallus of *E. lingulata*.

FIG. 11. Young spheroid *E. lingulata* sporeling undergoing oblique cell divisions.

appeared again, but were common in their generation.

Mating experiment results are given in Table I. Successful matings occurred between a typical filamentous thallus which produced the smaller biflagellate gamete and a spheroid form which produced the larger biflagellate gamete. The zygotes examined produced only filamentous forms. A random transfer of these forms gave the progeny listed in Table I for the first and successive generations. The progeny then began producing only their own morphologies, either filamentous or spheroid.

The results of the nuclear staining show that both typical and atypical morphologies had closely synchronized cell divisions (as in Fig. 10) which began to occur 1 h after the dark period had commenced. However, the orientation of these divisions differed during developmental stages (Figs 11 and 12). Spores from the typical filament produced the approximately 16-celled stage filament found in early development in other members of the Ulvales. Then transverse anticlinical walls were laid down as the young plant increased in girth. This species of *Enteromorpha* is characterized by having its cells arranged in longitudinal and transverse rows throughout (Kapraun, 1969), and oblique divisions did not take place until gamete or zoospore production in the adult (30 day) plant. As Figs 11 and 12 show, the spheroid thallus began oblique anticlinical cell divisions at an earlier developmental stage, and its thallus was a result of continuous divisions of this nature (Fig. 13).

Examples of both the typical and spheroid thallus type were isolated into axenic culture in artificial

TABLE 1. Three matings of sphere × filament performed with *Enteromorpha lingulata* (S = spheres, F = filament). First mating shows 1:1 ratio

	Mating No.					
	1		2		3	
	Phenotype S	Phenotype F	Phenotype S	Phenotype F	Phenotype S	Phenotype F
Sporophytes from P_1	0	1	0	1	0	1
Gametophyte progeny	7	185	74	133	10	157
Successive transfers*	195	13	24	0	5	0
Total F_1	202	198	98	133	15	157

* Spheroid progeny from filamentous phenotypes and filamentous progeny from spheroid phenotypes which appeared in later generations.

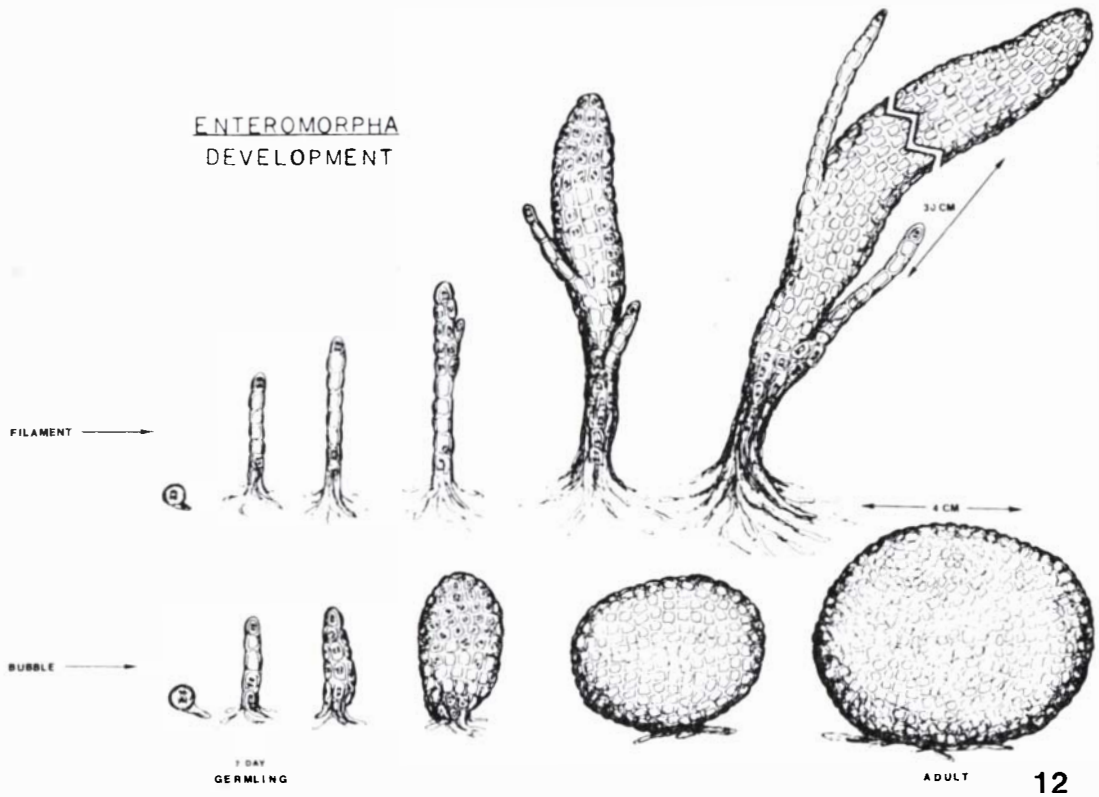

FIG. 12. Comparison of developmental stages of filament *vs.* sphere showing areas and orientation of cell division.

media. Their morphology was changed in that each plant became lumpy and warty in appearance in the artificial media (Figs 14 and 15). However, the basic spheroid and filamentous shapes were maintained through successive transfers by the production of biflagellate spores by the mother thalli. If the sporelings were transferred to natural sea water medium, they assumed the more characteristic spheroid and filamentous morphologies. The normal plant morphology as compared to field collected *E. lingulata* was maintained in axenic culture only if natural seawater was used in the medium. It was also noted that high doses of Streptomycin S (greater than 0·66 mg/ml) in liquid medium induced the formation of many spheroid thalli from one typical thallus (Fig. 16) but gametes produced by the spheres only gave rise to filaments in fresh von Stosch medium. Thus, the streptomycin-induced spheroidicity was not inherited.

The filter sterilized exudates from either typical or atypical thalli had no visible effect on axenic subcultures of the original axenic isolations, whether in natural or artificial media.

Discussion

The following observations provide evidence that the spheroid thallus is a mutation and not a culture phenomenon: (1) diploid plants (those producing quadriflagellate zoospores) from both mating and transfer experiments are never spheroid, (2) spheroid thalli only reproduce themselves and (3) axenic culture and media have no effect on the two basic typical and atypical morphologies. The mating experiment was also proof of mutation. The mixed results of the mating experiments, in which 1/3 were Mendelian ratio, must be examined carefully, however, because of inherent problems in dealing with mutant plants. Diploidization, single plants producing both haploid and diploid spores, and uniparental inheritance may have occurred in early experiments and could have added to the confusion in interpretation. Later crosses gave non-Mendelian ratios and in some cases, this may have been due to abnormalities relating to gamete production. The later mating experiments were also increasingly difficult because gametes produced by the spheroid

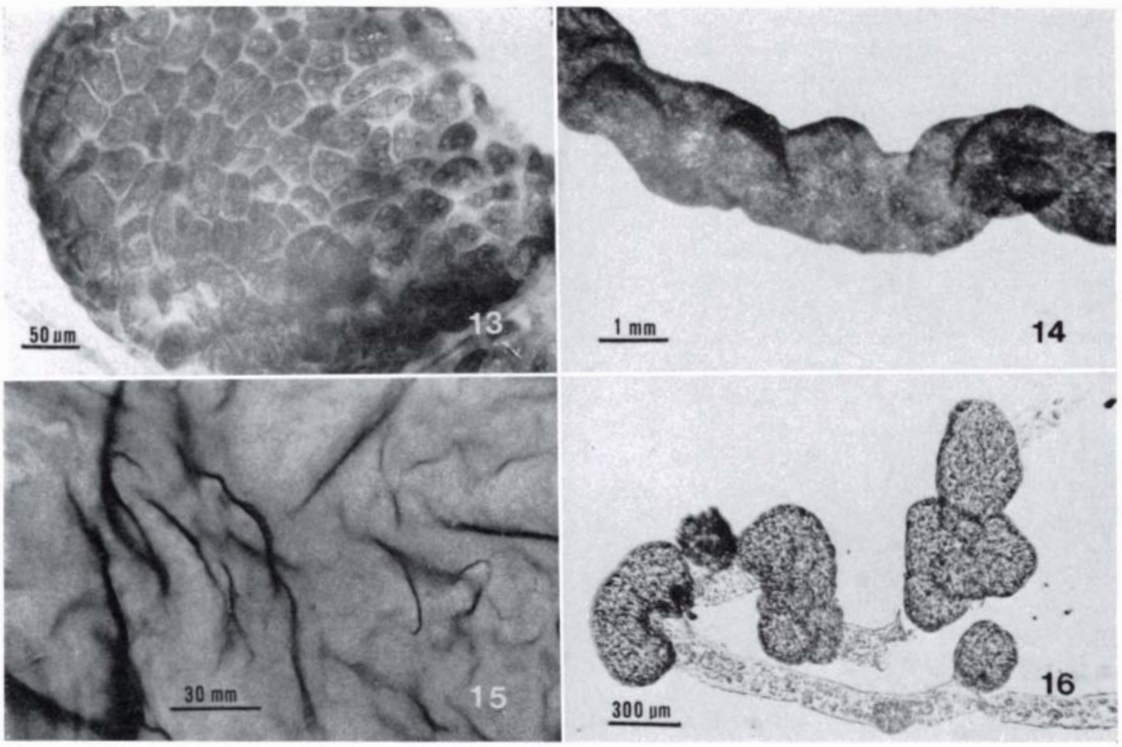

FIG. 13. Adult spheroid thallus of *Enteromorpha lingulata* showing disoriented cells from basal view.
FIG. 14. Abnormal appearance of filamentous thallus in axenic culture.
FIG. 15. Abnormal appearance of surface of spheroid thallus in axenic culture.
FIG. 16. Spheroid thallus formation on filament due to streptomycin treatment.

thallus appeared to lose their ability to fuse with the correct size gamete from filamentous thalli. Instead, the gametes tended to germinate parthenogenetically and spheroid thalli lost the ability to produce diploid spores. In view of the mixed results of the mating experiments, the atypical thallus has been influenced, at least in part, by mutation(s) in a chromosomal gene and probably by chromosomal-epichromosomal interaction.

The factors controlling cell division orientation in the *Enteromorpha* mutant are unknown, but, based on the mating experiments, appear to be influenced by chromosomal and epichromosomal genes, and are thus far phenotypic in only one sex. Streptomycin resistance in *Chlamydomonas reinhardtii* (Sager, 1954) is similar in these respects. *Chlamydomonas reinhardtii* plastids have also been indicated to have significant amounts of DNA (Chun, Vaughan & Rich, 1963; Sager & Ishida, 1963) and may be the vehicles of uniparental, non-Mendelian inheritance (reviewed by Adams *et al.*, 1976). In *Chlamydomonas*, however, isogametes contribute equally to the zygote, whereas in *Ulva mutabilis* (Fjeld, 1971) and *Enteromorpha lingulata* unequal contribution by anisogametes occurs. This would be advantageous for cytoplasmic or plastid influence. Also in *U. mutabilis*, the plastid of the smaller anisogamete disintegrates following zygote formation (Bråten, 1973), emphasizing the importance of the larger female gamete. Since the spheroid mutation of *E. lingulata* is indicated to have non-Mendelian characteristics, and because its plastid is inherited unequally toward the female and has adequate DNA, another approach to this problem will be to determine if the plastid is the source of the mutation.

A clue to the possible mechanism for influencing the direction of cell division may lie in the orientation of cell organelles. Prior to cell division in *Ulva mutabilis* (Løvlie & Bråten, 1970) the nucleus, plastid and vacuole reorient themselves. Plastid

orientation has been shown to be a diurnal rhythm in *Ulva* by Britz & Briggs (1976), where the plastids orient themselves along the sidewalls at night thereby greatly decreasing their light absorbance. The latter authors did not find this orientation phenomenon in several species of *Enteromorpha* tested, including *E. linza* (which has a flattened, *Ulva*-like thallus). The present authors found this diurnal rhythm in the spheroid mutation, but not in field collected *Enteromorpha lingulata* (unpublished data). If plastid orientation is tied to cell division orientation and if only flattened *Ulva*-type distromatic thalli exhibited this plastid phenomenon, then mutations occurring in this system (via plastid?) could cause *Ulva*-type thalli to become *Enteromorpha*-type thalli, and vice versa. In the latter case, however, it would seem to be more difficult for an *Enteromorpha* to gain the plastid orientation phenomenon through mutation than for an *Ulva* to lose it through mutation.

Other phenomena were observed in the cultures of *E. lingulata* spheroid and filamentous thalli which were not mutations, although they resulted in atypical thalli. Branch formation, monostromatic thallus formation and adventitious rhizoid formation by spheroid thalli were not inherited, although rhizoids were much reduced. Spheroidicity itself is not always mutational as the streptomycin abnormalities showed. The complex nature of inherited morphological characteristics of the Ulvales such as branch, rhizoid, filament and blade formation, cell division orientation and timing, and morphological variations in sex and generation must consist of many interactions between cellular and extracellular factors. These cannot be summarized by strict adherence to Mendelism, non-mutational variability or algal and microfloral exudates. This is the reason why great care must be taken in relying strictly on culture studies to determine the taxonomic affinities in the Ulvales.

More field studies similar to those of de Silva & Burrows (1973) are needed to clarify both systematic and taxonomic problems.

A striking characteristic of the spheroid thallus of *E. lingulata* is its morphological and developmental similarity to Fjeld's (1970) bubble mutant of *Ulva mutabilis*. As previously stated, the bubble mutant of *Ulva* was caused by disorientations of mitotic spindle apparatus in early developmental stages. Cell division orientation in the *Enteromorpha* mutant may have been also due to mitotic spindle orientations as evidenced by their similarities. In addition, the *Enteromorpha* mutant maintained its phenotype in axenic culture and in successive transfers of unialgal culture by producing the larger 'female' anisogamete.

The question of whether abnormal Ulvalean thalli occur in the natural environment has been raised by workers (Fjeld & Løvlie, 1976; Bonneau, 1977). Reports of them are rare, however. The morphologically globose *Collinsiella tuberculata* S. et G., considered by Scagel (1960) to be an abnormal stage or a rejuvenated basal portion of *Enteromorpha intestinalis* (L.) Link may be an example. *Collinsiella cava* (Yendo) Printz (or as *Ecballocystis cava* Yendo) has been considered similarly as an abnormal gametophyte stage of *Monostroma* or *Ulva* (Yendo, 1903; Chihara, 1958). Since cultured *Enteromorpha lingulata* has been found by the present author to grow poorly in seawater from Galveston, Texas, and by Kapraun in seawater from Port Aransas, Texas, it is probable that abnormal thalli of this species may occur in those localities.

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