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A chromosomal factor exerting a predetermining effect on morphogenesis in the multicellular green alga *Ulva mutabilis*

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1. SUMMARY

A new spontaneous mutant *bubble* (*bu*) with a marked effect on early morphogenesis in the multicellular green alga, *Ulva mutabilis* is described. It behaves as a recessive chromosomal mutant. The bu^+ gene product formed in heterozygous sporophytes exerts a predetermining effect on morphogenesis of haploid gametophytes with the *bu* genotype. It is suggested that the product of the bu^+ gene affects the orientation of the mitotic spindles during early development.

2. INTRODUCTION

Several examples are known in which development of a particular character is not determined by the genotype of the individual itself, but by genes present in the mother. Well-known examples are the direction of coiling in *Limnaea peregra* (Boycott *et al.* 1930) and the *grandchildless* character in *Drosophila subobscura* (Spurway, 1948). The present paper describes a chromosomal factor with a similar predetermining effect on the development of the gametophytes of a haplo-diplontic alga. Because of the small number of well-described cases, chromosomal genes with a predetermining effect on the progeny tend to be considered as oddities. The present results suggest that in *Ulva* such genes may be of significance in the genetic control of morphogenesis.

3. MATERIALS AND METHODS

The multicellular green alga *Ulva mutabilis* (Føyn) was first found on the south coast of Portugal in 1952 and has since been cultivated in the laboratory. The species is morphologically similar to *U. lactuca* and *U. thureti*, which also occur along the European coast (Føyn, 1958).

The life-cycle (Text-fig. 1) alternates between a haploid phase consisting of zoospores, gametophytes and gametes, and a diploid phase of zygotes and sporophytes. The zygotes develop into diploid sporophytes which form haploid zoospores by meiosis. The zoospores grow into haploid gametophytes of two different mating types. These are designated mt^+ and mt^- and are genetically determined. The gametophytes produce gametes by mitotic divisions and these usually fuse in

pairs to form zygotes which are heterozygous for mating type. If gametes do not fuse to form zygotes they will occasionally develop into haploid gametophytes identical to their parents. More often, however, there is a doubling of the chromosome number and diploid parthenosporophytes are formed which are homozygous for mating type, so homozygous diploids and haploids having the same genotype can be produced.

Gametophytes and sporophytes develop in the same way and are morphologically identical. During early development a single filament is formed by cell divisions having the mitotic spindles parallel to the long axis of the plant. When the filament consists of about 16 cells, the spindles are also formed at right angles to the long axis and the plant becomes cylindrical. The basal part, which is directed away from the light, differentiates into a holdfast consisting of giant stem cells and rhizoid cells. The distal part, pointing towards the light, becomes compressed and differentiates into a blade consisting of a double layer of cells.

Ulva mutabilis has spontaneously produced a number of morphological mutants, some of which have already been described (Føyn, 1959, 1960, 1961, 1962). The wild type and two of the mutants are used in this study.

The chromosomal mutant *Slender* (*Sl*) (Føyn, 1959; Løvlie, 1964) initially grows in the same manner as wild type, but the plant consists of not 16 but 30–60 cells when the orientation of the spindles changes. A holdfast is not formed. The rhizoids are poorly developed, giant stem cells do not develop and the plant grows into a long ribbon consisting of a double layer of cells which are morphologically similar to the blade cells of the wild type. The mutant grows faster than wild type, and it is semi-dominant. The Sl^+/Sl sporophytes are organized as wild type with blade and holdfast, but they grow faster and are more elongated.

The *bubble* mutant (*bu*) has not been described previously. The plants are hollow with a wall one cell thick and do not develop a holdfast with giant stem cells like the wild type. They can be spherical, elongated or elongated with a pointed end and having a few rhizoid cells (Plate 1). The crosses described below show that *bu* behaves as a recessive chromosomal mutant. The mutant does not develop into a long filament of cells like wild type or the mutant *Slender*. The spindle orientation may change immediately after the first division. The mutant also differs from the wild type in the time needed to develop into a fertile plant. The average time necessary for 20 *bu* gametophytes was 23 days, but 20 wild-type gametophytes took on the average 40 days.

Wild type and mutant plants were grown in Petri dishes containing 'Erdschreiber' medium (1000 ml sea water, 50 ml soil extract, 0·1 g $NaNO_3$ and 0·02 g $Na_2HPO_4.12\,H_2O$), with a light–dark cycle of 17 h light (4 a.m.–9 p.m.) and 7 h dark. The light source was from Phillips TL 40W/33 fluorescent lamps at an intensity of about 4000 Lux, and the temperature was kept at about 18 °C.

Almost the whole thallus may produce swarm cells in *Ulva*, and if this is not prevented the fertile plants are quickly spoiled. They are therefore stored at 5 °C, and when swarm cells are needed pieces of thallus are torn off and transferred to fresh medium and routine culture conditions. Three days later the swarm cells are

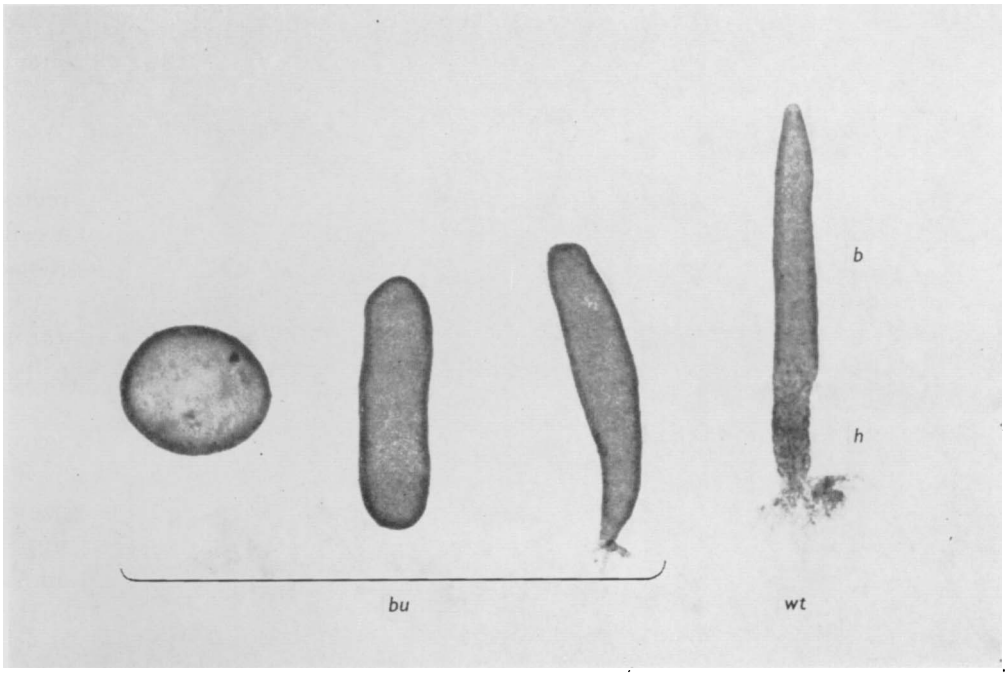

Plate 1. Three *bu* gametophytes illustrating the phenotypic variants of the mutant, and a wild-type gametophyte (*wt*) of *Ulva mutabilis*. All 14 days old. The wild-type gametophyte consists of a holdfast (*h*) with giant stem cells and rhizoid cells, and a primordial blade (*b*). 50 × .

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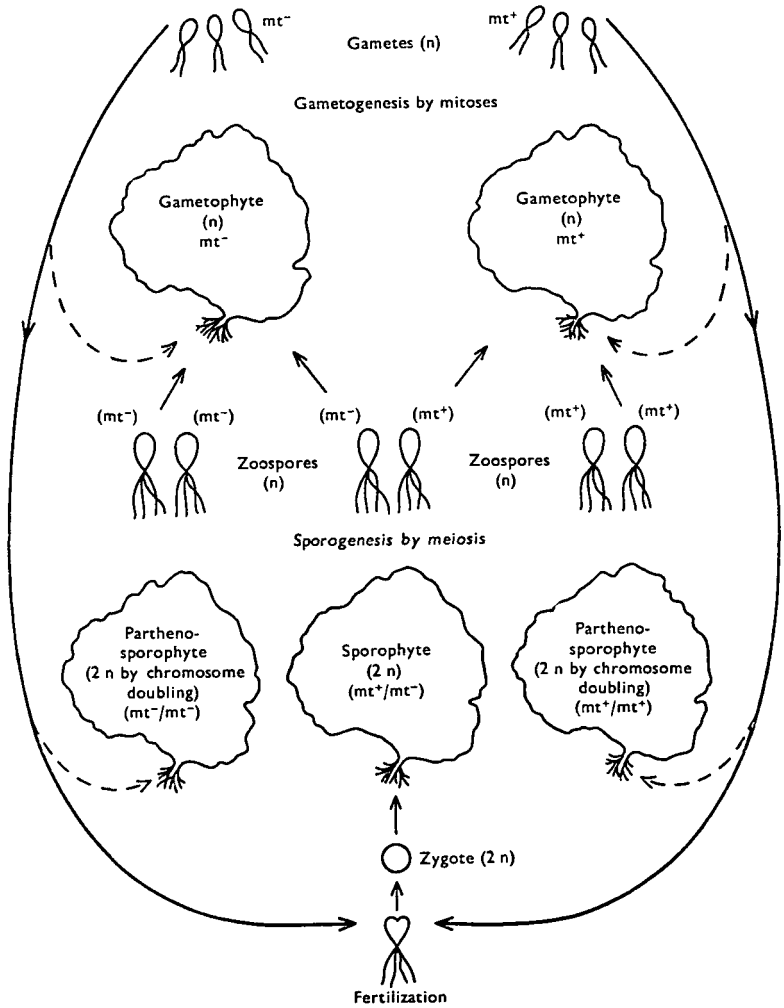
usually released from the fragments. Matings between gametes were made on microscope slides in front of the light source as described by Føyn (1958, 1959). Positively phototactic mt^+ and mt^- gametes were mixed in a drop of culture medium. After 10 minutes the uncopulated gametes had gathered on the light side of the drop while the negatively phototactic zygotes were on the dark side and could be transferred to Petri dishes with a pipette.



Text-fig. 1. Life-cycle of *Ulva mutabilis*. mt^+ and mt^--mating types.

4. RESULTS

The cross bu $mt^- \times bu^+$ mt^+ (*Table 1*)

The heterozygous sporophytes (bu^+/bu) from the cross $bu\ mt^- \times bu^+/mt^+$ were phenotypically like wild type, and both wild type and mutants were found among the haploid gametophytes formed by meiosis. However, the ratio between wild

type and mutant was rather unusual. Of 238 gametophytes found within a random area of a Petri dish only 35 (15 %) could be classified as mutants, but 181 (76 %) were phenotypically wild type. The phenotype of 16 (7 %) was intermediate between mutant and wild type, and the remaining 6 (3 %) had to be classified as irregular specimens.

Table 1. *The haploid gametophytes from the cross* bu mt$^-$ × bu$^+$ mt$^+$

	Phenotypes				
Genotypes	Wild type	Bubble	Inter-mediate	Irregular	Total
bu$^+$	123	—	—	3	126 (53 %)
bu	58	35	16	3	112 (47 %)
Total	181 (76 %)	35 (15 %)	16 (7 %)	6 (3 %)	238

Thallus fragments from each of the 238 gametophytes were then allowed to form gametes which were not mixed, but developed parthenogenetically. The parthenosporophytes were then examined for phenotype. Of the 181 gametophytes with wild-type phenotype only 123 gave wild-type progeny, the remaining 58 being mutant. The 51 gametophytes classified as mutants or intermediates all gave mutant progeny. Three of the irregular plants gave mutant progeny and three gave wild type.

Judging from the phenotypes of the parthenogenetically formed progeny, the numbers of *bu*$^+$ and *bu* gametophytes were 126 and 112 respectively, indicating a 1 : 1 ratio, i.e. *bu* behaves as a chromosomal factor.

However, only 35 (31 %) of the 112 *bu* gametophytes were phenotypically bubble. 58 (52 %) showed a wild-type phenotype, and 16 (14 %) classified as intermediate, showed a tendency towards this phenotype. This suggests that the *bu*$^+$ factor of the *bu*$^+$/*bu* sporophyte had exerted a predetermining effect on the gametophytes.

The time necessary to develop from zoospores to fertile plants can be correlated with the morphology of the *bu* gametophytes. The average time for those with mutant phenotype was 25 days, for those with intermediate and wild-type phenotypes 37 days and 41 days respectively.

The cross bu mt$^+$ × bu$^+$ mt$^-$ (*Table 2*)

Table 2 shows the results for the cross using reciprocal mating types. The results are essentially the same as before. The *bu*$^+$/*bu* sporophytes were phenotypically wild type. Of 149 randomly sampled gametophytes 74 were genotypically *bu*, but only 21 (28 %) of the 74 *bu* gametophytes had mutant phenotype. 11 (15 %) had intermediate phenotypes, and 39 (53 %) were phenotypically wild type.

Table 2. *The haploid gametophytes from the cross* bu $mt^+ \times bu^+ mt^-$

	Phenotypes				
Genotypes	Wild type	Bubble	Inter-mediate	Irregular	Total
bu^+	73	—	—	2	75 (50 %)
bu	39	21	11	3	74 (50 %)
Total	112 (75 %)	21 (14 %)	11 (7 %)	5 (3 %)	149

The cross bu Sl^+ $mt^- \times bu^+$ Sl mt^+ (*Table 3*)

The heterozygous sporophytes (*bu*$^+$/*bu*, *Sl*$^+$/*Sl*) had the same phenotype as sporophytes from crosses between *Sl* and wild type (see Materials). The phenotypes of 280 randomly sampled gametophytes were: 101 (36 %) wild type, 80 (29 %) slender, 42 (15 %) bubble, 12 (4 %) intermediate between wild type and bubble, 36 (13 %) intermediate between slender and bubble and 9 (3 %) irregular.

Table 3. *The haploid gametophytes from the cross bu* Sl^+ $mt^- \times bu^+$ Sl mt^+

	Phenotypes						
Genotypes	Wild type	Slender	Bubble	Inter-mediate between wild type and bubble	Inter-mediate between slender and bubble	Irregular	Total
bu^+ Sl^+	65	—	—	—	—	1	66 (24 %)
bu^+ *Sl*	—	73	—	—	—	4	77 (28 %)
bu Sl^+	36	—	23	12	—	1	72 (26 %)
bu Sl	—	7	19	—	36	3	65 (23 %)
Total	101 (36 %)	80 (29 %)	42 (15 %)	12 (4 %)	36 (13 %)	9 (3 %)	280

Fragments from the gametophytes were allowed to form gametes and the phenotypes of the parthenosporophytes derived from them were examined. 65 of the 101 gametophytes with wild-type phenotype gave wild-type progeny and 36 gave bubble. 73 of the 80 gametophytes with slender phenotype gave slender progeny and 7 gave bubble. The gametophytes classified as bubble (42) and intermediates (12 + 36) gave bubble progeny, and of the 9 irregular specimens 1 gave wild type, 4 gave slender and 4 gave bubble progeny. In all, 66 gametophytes gave wild type, 77 gave slender and 137 gave bubble progeny. The genotype of the 137 gametophytes with bubble progeny was determined by backcrossing to *Sl* gametophytes. The resulting sporophytes having the slender phenotype indicated that the gametophyte in question had the *bu Sl* genotype, whereas sporophytes with a phenotype like *Sl*$^+$/*Sl* sporophytes indicated the *bu* Sl^+ genotype. 72 gametophytes were

bu Sl$^+$, namely 23 of the 42 with bubble phenotype, the 36 with wild-type phenotype, the 12 with phenotypes intermediate between wild type and bubble, and 1 of the irregular specimens. 65 gametophytes were *bu Sl*, namely 19 of the 42 with bubble phenotype, the 7 with slender phenotype, the 36 with phenotypes intermediate between slender and bubble, and 3 of the irregular specimens. The results indicate a 1:1:1:1 distribution between *bu*$^+$ *Sl*$^+$, *bu*$^+$ *Sl*, *bu Sl*$^+$ and *bu Sl* and accordingly that *bu* and *Sl* segregate independently of each other. The bubble phenotype of *bu Sl* plants shows that *bu* is epistatic to *Sl*.

The results suggest, as in the previous crosses, a predetermining effect of the *bu*$^+$ gene of the heterozygous sporophyte on the gametophyte progeny. In relation to the *bu Sl* gametophytes this effect made it possible for the *Sl* gene to be expressed in spite of *bu* being epistatic, namely the *bu Sl* gametophytes with slender phenotype or phenotypes intermediate between slender and bubble. Since the heterozygous sporophyte (*bu*$^+$/*bu*, *Sl*$^+$/*Sl*) differed from wild type phenotypically, the predetermining effect may be independent of the phenotype of the sporophyte (provided the *bu*$^+$ factor is present).

The proportion of both the *bu Sl*$^+$ and the *bu Sl* gametophytes which seems to be influenced by the *bu*$^+$ factor of the parent is about 60–70 % and is therefore independent of the *Sl*$^-$ factor. However, the *Sl*$^-$ factor seems to influence the degree of suppression of the *bu* character. 50 % of the *bu Sl*$^+$ gametophytes had a wild-type phenotype, and 11 % of the *bu Sl* gametophytes were phenotypically slender, i.e. the *bu* character was completely suppressed in 50 % of the *bu Sl*$^+$ gametophytes, but for the *bu Sl* gametophytes the corresponding proportion was only 11 %.

5. DISCUSSION

The delayed effect of a chromosomal gene on individuals of the next generation has until now been demonstrated only in oogamous species. In these, the transfer of the gene product seems to take place only through the cytoplasm of the egg and has therefore been described as a maternal effect. The effect of the *bu*$^+$ gene in *Ulva mutabilis* is similar to the maternal effect. However, as the alga is a haplodiplont and the discovery has been made in connexion with the formation of undifferentiated zoospores, it is perhaps better to speak about a predetermining effect of the *bu*$^+$ gene and include in the general concept of predetermination by chromosomal genes similar examples which have previously been described as maternal in oogamous species.

The *bu* mutant is usually unable to carry out normal morphogenesis. It consists mainly of one cell type and the plant is not differentiated into a blade and holdfast with rhizoid cells and giant stem cells like the wild type. However, the mutant has not lost the capacity to develop normally. Under the influence of the *bu*$^+$ product transferred to the zoospores from the heterozygous sporophytes, about 70 % of the gametophytes with the *bu* genotype were wholly or partly wild type phenotypically.

At present it is not clear why the predetermining effect of the *bu*$^+$ gene leaves 30 % of the *bu* gametophytes unaffected. In *Drosophila melanogaster* the pre-

determining effect of the *maroon-like* character is dependent on the age of the culture. Only flies which hatch during the first 4–5 days show any predetermination (see Chovnick & Young, 1968). A similar explanation cannot be applied to *Ulva* as the zoospores are released simultaneously and the developing gametophytes are exposed to identical conditions. It is possible that the bu^+ product is made before sporulation in such small quantities that only 70 % of the *bu* zoospores contain it. If the bu^+ product is made in large amounts, there would have to be some mechanism causing an unequal distribution.

Generally, for normal growth to occur the products of some genes must be available throughout development, while the products from others are necessary only during particular periods. The quantity of the bu^+ product in the *bu* gametophytes is presumably limited, possibly because the zoospores which transfer the product from the heterozygous sporophytes are only about 10 μ in length (Føyn, 1958). The idea of a limited quantity is also supported by 30 % of the *bu* gametophytes being unaffected by the bu^+ factor. As 50 % of the *bu* gametophytes had a normal wild-type phenotype and were not intermediate it seems unlikely that the bu^+ product is essential during the late periods of development. It is more likely that the bu^+ product is essential only during the initial phase of development. The fact that the *bu* mutant deviates from the wild-type pattern at the very beginning of development supports this idea. In wild type the spindles are always parallel to the long axis of the plant until about the 16 cell stage, so that the wild type starts its development as a filament consisting of a single row of cells. In the mutant the spindles are not necessarily oriented in this way and a filament is not usually formed. The formation of a single row of cells may therefore be necessary for the later differentiation of the holdfast with giant stem cells and rhizoid cells, which is characteristic of wild type, but absent in the *bubble* mutant. If this is so, the bu^+ product may affect the orientation of the spindles of the early divisions.

The mutant *Slender* also starts its development as a filament. The epistasy of the *bu* mutation over *Slender* is in agreement with the idea that the bu^+ gene participates in the regulation of the early cell divisions. The *Slender* germling has about twice as many cells as wild type when the orientation of the spindles changes direction, and this may explain the difference in response to the transferred bu^+ product in *bu* Sl^+ and *bu Sl* gametophytes (Table 3). Of the 72 *bu* Sl^+ gametophytes 36 (50 %) developed a wild-type phenotype while 12 (17 %) developed phenotypes intermediate between wild type and the *bu* mutant. On the other hand, only 7 (11 %) of the 65 *bu Sl* gametophytes had a slender phenotype, and 36 (55 %) were intermediate between the *bubble* and *Slender* mutant. If the bu^+ gene has a predetermining effect on the spindle orientation of the early mitoses it would be more likely that a wild-type phenotype would be formed in a *bu* Sl^+ gametophyte than the slender phenotype in a *bu Sl* gametophyte. In the former a filament of about 16 cells seems to be necessary, while in the latter, one consisting of 30–60 cells is needed.

It is evident from the experiments on sea urchins and frogs that the first phase of development may be under the influence of gene products accumulated in the egg before fertilization (see Gross, 1968). In these animals a detailed genetical analysis

may prove to be difficult, because mutations which influence the early stages of development are presumably lethal. However, the present study suggests that such genes may be available for a genetical analysis in multicellular algae.

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