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# THE INHERITANCE OF SPONTANEOUS PIGMENT MUTATIONS IN CHONDRUS CRISPUS STACKH. (RHODOPHYCEAE).[1]

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Pigmentation mutants of the marine red alga *Chondrus crispus* were collected from intertidal populations and genetically characterized in culture. These mutants had various shades of green pigmentation, bright green at one extreme and brownish green at the other. Of 9 mutants characterized, 6 were found to have strict maternal inheritance whereas the remaining 3 yielded classical Mendelian transmission ratios. Thus both nuclear and non-nuclear, presumably chloroplast, mutants have been identified. One of the Mendelian mutants is very cold-sensitive, being bright green at 5°C and brownish-green at 20°C. It is anticipated that some of the characterized mutants will prove useful for future studies on the biology of *C. crispus*.

Des mutants de pigmentation de l'algue rouge *Chondrus crispus* ont été récoltés dans des populations intertidales et leur génétique a été décrite en culture. Ces mutants sont pigmentés en vert de différents nuances allant du vert clair au vert brunâtre. Des 9 mutants décrits, 6 ont une hérédité strictement maternelle alors que les autres ont une hérédité mendélienne classique. On a ainsi identifié et des mutants nucléaires et des mutants non-nucléaires, ces derniers étant probablement des mutants chloroplastiques. Un des mutants mendéliens est trés sensible à la température: il est vert clair à 5°C et vert brunâtre à 20°C. On s'attend à ce que certains de ces mutants soient utiles pour des études ultérieures de la biologie de *C. crispus*.

## Introduction

*Chondrus crispus* (Irish moss) is a common and commercially harvested red alga in Atlantic Canada. It is, in fact, the only alga in the region that supports a substantial industry, harvests of all other algal species being small by comparison. It is not surprising, therefore, that a considerable amount of research has been devoted to studies on the biology of *C. crispus* in recent years. (Bibliographies of this research have been compiled. See Campbell 1973; Mackey & Taylor 1979).

A workshop on *C. crispus* was convened in 1972 to review what was known about the alga and to focus attention on areas that needed additional study (Harvey & McLachlan 1973). Although much has been learned in the decade following that workshop, many questions remain unanswered or only partly answered. Thus it is likely that research on this alga will still continue for some time.

Up to the present time, most, if not all, research has utilized normal wild plants or populations, in particular a few vegetatively propagated clones of which the Atlantic Research Laboratory's strain "T4" (Shacklock et al. 1973) is perhaps the best known. For some of the anticipated future studies it is likely that mutant plants, in addition to normal plants, will be a valuable asset. Such has been the case in numerous areas of biological and biochemical research, including our own studies on other red algae, namely *Gracilaria tikvahiae* and *Palmaria palmata* (van der Meer 1981; van der Meer & Todd 1980). Because *C. crispus* has a long life

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cycle that requires almost 2 years to complete even in optimal culture conditions (Chen & McLachlan 1972), a considerable amount of time is required to characterize a mutant strain once a need has been identified. This presents a serious deterrent to the use of mutants. Accordingly, we have decided to characterize some mutants immediately in the anticipation that they will prove useful as markers, and will be ready when needed.

## Materials and Methods

Mutant and wild-type fronds were collected at Ketch Harbour and at Finck Cove, both in Halifax County, Nova Scotia. Fronds with altered pigmentation were reasonably common, a single foray often yielding 10 or more mutants. In early spring, when normal fronds are well pigmented, even small mutant sectors can be detected. In summer, when many plants become pale and greenish due to their physiological adaptations to summer growing conditions, mutants are harder, but not impossible, to detect. One of the mutants was obtained from a green sector that arose spontaneously on the T4 clone growing at the ARL marine station.

Apical segments were grown in culture with repeated brushing and trimming until unialgal cultures were established. In some cases the reproductive phase of the plants was known from the onset because the collected plants were fertile; in others, this had to be established in culture. Crosses were made by co-culturing male fronds with unfertilized female fronds grown in solo culture for at least 3 months. In cases where the original mutant frond was tetrasporophytic, male and female plants had to be obtained from tetraspores before genetic testing could begin.

Growth conditions were variable and often not optimal. Plants were usually grown at 20°C, but at times they had to be moved to 5°C to help control a rotting disease which appeared in some cultures. Light intensity varied from 30 to 70 $\mu Em^{-2}s^{-1}$ and included both long and short daylengths. Culture medium was usually SWM-3 without added soil or liver extract (McLachlan 1973), but in some cases Tris buffer was omitted to reduce bacterial growth. Characterization of these mutants was done as a sideline over a period of about 4 years which partly accounts for the culturing variability mentioned. However, this variability in no way affects the validity of the genetic determinations. Under these growing conditions, each phase of the life cycle required about 1 year to reach maturity, and thus at least 2 years were necessary to study the transmission of a mutation through a complete sexual life cycle.

It should be noted here that the strain T4, whose sexuality had not been firmly established at the time, was found to be a male plant when the clone at the marine station produced abundant spermatia during the autumn and winter of 1979. Interestingly, Guiry, working with a subclone of T4 in Ireland, independently discovered at much the same time that T4 could be induced to produce spermatangia in culture (Guiry 1981).

## Results and Discussion

Genetic data have now been collected for 9 different mutant plants, all with green or greenish phenotypes. All were spontaneous mutations, 8 of them derived from wild intertidal plants, and 1 from the clone T4 at the ARL marine station. Characteristics of these plants are summarized in Table I. Note that both tetrasporophytes and gametophytes were represented in the original collection.

**Table I.** Characteristics of the mutants.

| Mutant | Color | Phase and Sex | Size of Original Mutant Sector | Sorting-out Pattern of Mutant Tissue |
|---|---|---|---|---|
| M-1 | brownish green | T | whole plant | unknown |
| M-2 | bright green | T | partial frond | gradual |
| M-3 | green | M (T4) | whole frond[a] | unknown[a] |
| M-4 | bright[b] green | M | whole frond | rapid |
| M-5 | yellowish green | M | partial frond | gradual |
| M-6 | pale green | M | partial frond | gradual |
| M-7 | bright green | F | whole frond | unknown |
| M-8 | green | F | whole plant | unknown |
| M-9 | brownish green | F | partial frond | gradual |

Abbreviations: T = tetrasporophyte, M = male, F = female

[a]The T4 green clone had arisen years ago and was maintained vegetatively but we have no record for its sorting out.

[b]This color is cold-sensitive. At 5°C it is bright green, but at 20°C it is brownish-green.

Note also that most of the mutants were found as single fronds or sectors of otherwise normal plants. This makes it likely that each is an independent mutation. The absence of bright green complete plants is consistent with the interpretation that most of these mutant phenotypes have a competitive disadvantage, and are not readily propagated in the natural environment. Table I also records, where known, the sorting-out pattern of mutant from wild-type tissue. The sorting out of a nuclear gene would, in most instances, be expected to occur more rapidly than sorting out of a chloroplast gene. Thus from the sorting-out pattern it is possible to predict quite accurately whether a mutation will exhibit a Mendelian or a non-Mendelian transmission pattern.

The results from crosses are summarized in Table II. A control cross between wild-type male and female stocks produced only normal wild-type progeny, in-

dicating that these stocks could be used in crosses to characterize the mutants. The first 2 mutants tabulated were found as tetrasporophytes and although they differed greatly in color, they exhibited identical non-Mendelian transmission in crosses. In both cases, all gametophytes from the original tetrasporophytes had the same mutant phenotype as their parent. Male and female gametophytes were derived from each tetrasporophyte and were crossed reciprocally with the wild type. When mutant females were used, the resulting tetrasporophytes and subsequent gametophytes were mutant. However, when the mutant served as male parent, the mutation was not transmitted. In these crosses, the male's color phenotype was never detected among the thousands of sporelings obtained. These results are identical to those obtained for non-Mendelian mutations of another red alga, *Gracilaria tikvahiae* (van der Meer 1978), which also exhibited a strict maternal transmission pattern.

Mutant males M-5 and M-6 behaved like the males just mentioned and failed to transmit their mutant phenotypes even though these were stable during vegetative growth. Mutant females M-8 and M-9 behaved like the females above in that their diploid progeny had mutant phenotypes, and in that there was no segregation among the $F_1$ gametophytes. These 4 mutations were also non-Mendelian.

**Table II.** Summary of crossing results.

| Parents (♀ x ♂) | Phenotypes of the Tetrasporophytes | Phenotypes of the $F_1$ Gametophytes |
|---|---|---|
| *Control* | | |
| wt x wt | wt | all wt |
| *Crosses to wild type* | | |
| Unknown parents | brownish green | all brownish green |
| M-1 x wt | brownish green | all brownish green |
| wt x M-1 | wt | all wt |
| Unknown parents | bright green | all bright green |
| M-2 x wt | bright green | all bright green |
| wt x M-2 | wt | all wt |
| wt x M-3 (T4) | wt | 54 wt and 37 green |
| wt x M-4 | wt | 115 wt and 123 green |
| wt x M-5 | wt | all wt |
| wt x M-6 | wt | all wt |
| M-7 x wt | wt | 42 wt and 40 bright green |
| M-8 x wt | green | all green |
| M-9 x wt | brownish green | all brownish green |
| *Other crosses* | | |
| M-9 x M-4 | brownish green | 247 brownish green and 223 bright green |
| M-7 x M-3 | wt | no data |

Abbreviation: wt = wild type

The remaining 3 plants, 2 males (M-3 and M-4) and a female (M-7), had Mendelian transmission of their mutant phenotypes. In each case the mutation was not expressed in the hybrid $F_1$ tetrasporophyte and reappeared in half of the $F_1$ gametophytes, a pattern characteristic of a recessive nuclear gene.

Only a few crosses have been made among the mutant stocks themselves. Two, however, are listed in Table II. One cross was between a non-Mendelian green female and a Mendelian green male. The results were consistent with expectations as all progeny were green, but the influence of the recessive Mendelian gene was seen in the segregation pattern of the $F_1$ gametophytes. The final cross, between 2 recessive Mendelian mutations, showed complementation in the $F_1$ tetrasporophyte indicating that these mutations are in different cistrons.

Mutant M-4 is unusual when compared with the rest of the collection. Although the color of all the plants can vary somewhat with culture conditions, mutant M-4 is unique in that its color shows a pronounced cold sensitivity. It was found in late winter as a bright green mutation and that color was maintained at 5°C in culture. However, when transferred to 20°C, the color became brownish green, much closer to wild type, although still recognizably mutant. It reverts to bright green upon return to 5°C. The entire plant changes color, not just the newly synthesized tissue.

In summary, it is clear that spontaneous mutations are quite readily obtained both for nuclear and non-nuclear, presumably chloroplast, genes. Both types can be found in the field, although the non-Mendelian type appears to occur more frequently. Only the length of the life cycle presents a problem for genetic analysis, all other factors being straightforward. The sorting-out pattern of mutant tissue was found to be a reliable predictor for the type of mutation present (i.e., nuclear vs. cytoplasmic), and while perhaps not perfect, could be used to select for one kind of mutation or the other.

Having characterized just 9 mutants it is clear we have only begun to scratch the surface of what could be done. Unfortunately the long life cycle of *Chondrus* makes genetic analysis of this alga tedious. Perhaps it is enough to have a few characterized mutant stocks available as tools for future studies. On the other hand, some of the mutants themselves, for example the cold-sensitive green mutant, might well prove interesting for biochemical or physiological studies, which stimulates the desire to have even more mutants. Ultimately these are decisions each investigator will have to make for himself.

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