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Causes and implications of intra-clonal variation in *Gracilaria chilensis* (Rhodophyta)

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Abstract

Strain selection studies in *Gracilaria chilensis* detected significant levels of intra-clonal variation. These findings motivated more detailed studies on the causes and implications of intra-clonal variation in these and other red algal species. Our results indicate that intra-clonal variation is common among replicated units (e.g.: carpospores and ramets) of several red algal species and suggest that a larger data base probably will show the occurrence of various kinds of intra-clonal changes, differing in frequency of occurrence and magnitude of phenotypic expression. It is likely also that different species would exhibit different amounts of variation. Four types of factors may cause intra-clonal variation: (1) physiological or developmental differences among ramets, (2) localized pathogen infections, (3) several kinds of genetic changes, and (4) sporeling coalescence. Intra-clonal variation among ramets: (1) increases the possibility of genet survival, (2) explains the origin of morphological and physiological differences among ramets of a given genet, (3) explains the large population variation found in many clonal species and (4) suggests that strain selection of some economically important seaweeds should be thought of as a fairly continuous process due to the instability of some of these clones.

Introduction

Selection of superior strains is regarded as the next necessary step following the development of successful farming methods for any given algal species. Over the last 15 years, close to 30 studies have attempted strain selection in about a dozen seaweed species, including taxa in the red algal genera *Chondrus*, *Gigartina*, *Gracilaria* and *Kappaphycus* (see Santelices (1992) for a review).

By 1988, field farming of *Gracilaria chilensis* was gradually replacing the gathering of wild crops in Chile. Since 1983 the relative importance of farmed *Gracilaria* has steadily increased from 0.6 to 38.8% of the total crop and several factors suggested that *Gracilaria* farming was likely to increase further in the future (Santelices & Ugarte, 1990). As a result, from 1988, the first attempts to select superior strains of *Gracilaria chilensis* were made in this country. Agronomically desirable traits were defined, followed by experimental evaluations of the inter- and intra-specific variation with respect to these traits. Results suggested enough intra-specific differences as to attempt strain selection (Santelices & Ugarte, 1990).

Experimental results also showed significant intra-clonal variation in *Gracilaria chilensis*. Plant portions derived from the same clone and incubated under similar conditions frequently exhibited dissimilar growth responses (Santelices, 1992; Santelices & Varela, 1993a). Changes in the selected character over successive generations of cuttings from the same original fragment also were evident. These findings motivated more detailed studies on the patterns of intra-clonal variation in *Gracilaria chilensis* and experimental studies on the factors inducing such variation. This contribution summarizes results gathered from such studies combining previous findings with new data obtained in spore coalescence experiments.

1. Seaweeds as clones and intra-clonal variation

Any organism that grows and propagates by self-replication of genetically identical units can be considered a clone (Cook, 1985; Jackson *et al.*, 1985). The units forming the clone are known as ramets and they can function and survive on their own if separated from one another by natural processes or by injury. A genet is the sum of all ramets derived from a single zygote.

Many of the red seaweeds studied in strain selection processes, including *Gracilaria*, can be considered clonal organisms as they propagate by self-replication of genetically identical units (Santelices, 1992) and general principles learnt from the comparative studies of clonal organisms in the animal and plant kingdoms should therefore apply to them. Especially pertinent to the strain selection practices, are the data suggesting that significant phenotypic differences among ramets derived from a single genet are to be expected in most clonal organisms (Bonga & Durzan, 1985; Jackson *et al.*, 1985; Harper *et al.*, 1986). Genetic, physiological and developmental differences among ramets derived from a single genet, as well as pathogens differentially affecting some ramets within a clone, are recognized as the most important factors that might induce significant intra-clonal variation in the phenotypic expression (Buss, 1985; Harper, 1985; Silander, 1985; Watkinson & White, 1986).

2. Intra-clonal variation in *Gracilaria chilensis*

The life history of species of *Gracilaria* exhibits two kinds of mitotically replicated units. One kind is represented by the carpospores, which are dispersal units, originating from a single gametic fusion (Fredericq & Hommersand, 1989). The other kind are the branches (ramets) of gametophytes and sporophytes which are derived from mitotic divisions of a single genet.

Experimental incubation of populations of sporelings, each grown under similar culture conditions and derived from carpospores shed by the same cystocarp exhibited significant differences in growth (Fig. 1), attesting for significant intra-clonal variation among this type of mitotically replicated units (Santelices & Varela, 1993a). Significant differences occurred among young (30 days old) as well as older (180 days old) sporelings.

In adult thalli, branches (ramets) derived from the same thallus (genet) and grown under similar conditions also exhibited significant variation in growth rates and morphology (Santelices & Varela, 1993a). In this case, however, the variants are individual ramets and the lack of replication does not easily allow statistical testing through one way or multiway ANOVA. Data have been tested as significant individual departures from the confidence limits of the population mean value. In the case of *Gracilaria chilensis*, experimental ramet incubation under controlled conditions indicated that biomass increments were a function of initial weight as long as the algal biomass was maintained within the carrying capacity of the culture medium. The magnitude of the departure of extreme data points (arrows in Fig. 2) could then be tested against the confidence limits determined by the regression equation.

Using the above method, significant intra-clonal variation in growth rates of ramets has been detected

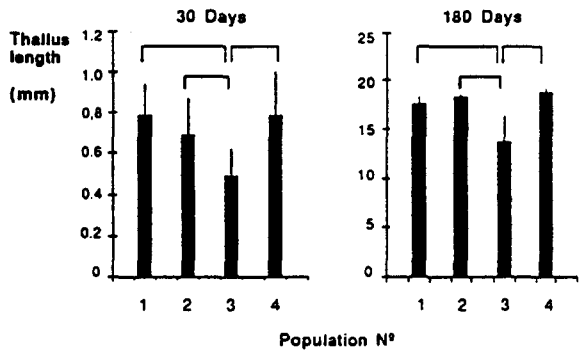

Fig. 1. Growth differences among populations of sporelings derived from carpospores shed within 48 h by a single cystocarp of *Gracilaria chilensis*. Elongation rates were measured after 30 and 180 days of incubation under controlled laboratory conditions. Bars are standard errors. Brackets indicate significant differences (ANOVA followed by Tukey test; $p<0.05$). Data and methods as in Santelices and Varela (1993a).

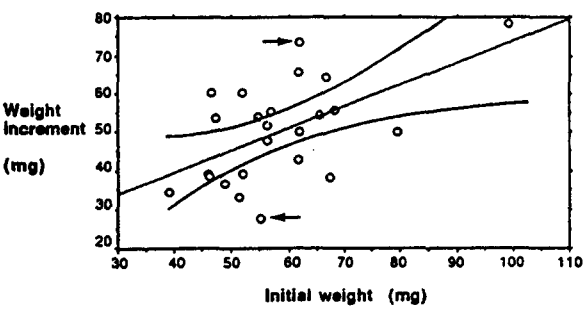

Fig. 2. Weight increments of ramets of *Gracilaria chilensis* as a function of initial weight after 30 days of incubation under controlled conditions. Arrows indicate extreme data points. The value of the regression equation is $y = 16.973 + 0.569x$; $r^2 = 0.305$; $p = 0.05$. Lines above and below the sample regression line are the confidence limits (95%). Data and methods as in Santelices and Varela (1993a).

in several clones and strains of *G. chilensis* from different geographic origins along Chile. In these experiments, special care has been taken to handle all ramets in a given experiment in the same way and to provide identical growth conditions to all replicates in order to reduce differences in the micro-environment around the ramets. In addition, physiological or developmental differences among ramets have been reduced as much as possible by the use of ramets of equivalent position along the axis, by selecting a similar order of branching and a similar portion of the branch, and by using ramets containing a similar number of branchlets. In spite of all these procedures, intra-clonal variation was common in our experimental results.

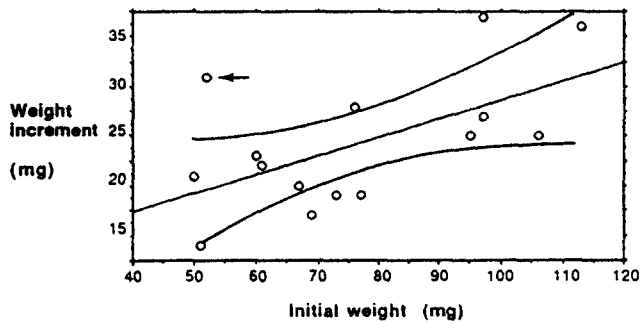

Fig. 3. Weight increments in ramets of *Gymnogongrus furcellatus* as a function of initial weight after 30 days of incubation under controlled conditions. Arrows indicate extreme data points. The value of the regression equation is $y = 9.938 + 0.188x$; $r^2 = 0.344$; $p = 0.02$. Lines above and below the sample regression line are the confidence limits (95%). These are new experimental results using the method described in Santelices and Varela (1993a).

3. Intra-clonal variation in order Rhodophyta

A review of the literature indicates that intra-clonal variation, among carpospores and among ramets, has been seen and described by previous authors studying red algae. Working with three species of *Gracilaria*, van der Meer and Zhang (1988) reported several cases of unstable mutations affecting pigmentation. Genetic reversions occurred during all phases of the life cycle of the species, including developing carposporophytes. Thus, some cystocarps that formed on green portions of green female thalli developed masses of reddish carpospores in addition to the green ones.

Among adult thalli, pigment or morphological variants have been reported to arise spontaneously in experimental or pilot culture of several species of *Gracilaria*, *Chondrus* and *Kappaphycus* (see review by Patwary & van der Meer (1992)). Working with *Gracilaria chilensis* (as *G. secundata*), (= *G. chilensis*), Lignell and Pedersén (1989) found that wild type species spontaneously differentiated into as many as eight morphologically different forms which differed from each other in frond thickness, branch frequency and agar composition. More recently, M. Jimenez del Río (pers. comm.) reported the formation of 3 morphological and pigment variants within 6 months of cultivation of *Gracilaria cornea*.

Although the above cases are obvious examples of intra-clonal variation in red seaweeds, these changes differ in magnitude from the growth differences detected in *Gracilaria chilensis* by Santelices and Varela (1993a). This last type of variation does not necessarily express itself in major pigment or morphological changes but rather in more subtle and frequent changes in growth or performance. Normally it requires controlled experimental conditions and statistical analysis to be detected. Since the literature generally contains reduced data, it is difficult to assess the frequency of this type of variation. However, the original data published by Neish and Fox (1971) when selecting strains of *Chondrus crispus* point to a similar variation. In their tank experiments, pieces of a given clone (T-4 or 0-6) were used to increase the number of replicates or to replace other plants that were dying. Plant portions derived from the same clone often exhibited dissimilar growth rates, even when incubated in the same tank. Ongoing research with other red algae (Fig. 3) also document the occurrence of these more subtle and frequent variants in growth performance. A larger data base would probably show the occurrence of various kinds of intra-clonal changes, differing in frequency of occurrence and in the magnitude of phenotypic expression. From the data so far gathered it seems likely that different species would exhibit different frequencies of changes.

4. Causes of intra-clonal variation

Four types of factors may cause intra-clonal variation in seaweed species (Santelices, 1992). Only two of these types of factors seem to have a genetic basis and are expected to propagate in successive generations of cuttings from the original variants. The other two factors are not genetically based and the variability induced by them is likely to disappear in successive generations of cuttings.

(a) Physiological and developmental factors

Differences in morphology or performance among ramets of a given clonal species might be induced by a diverse array of physiological or developmental factors, such as age of the ramet, distance from the tip, content of endogenous growth substances and others (Cook, 1985). Ongoing research has detected one such response in some strains of *Gracilaria chilensis* (Fig. 4). A finely-branched strain collected in Niebla, near Valdivia, in southern Chile (39 °48′S 73 °24′W), increases significantly the number of third order branches when the apical 1 cm of fourth order branches are removed. This response, which may be compared to the apical dominance responses of land plants, might explain some of the intra-clonal variations in branching patterns often observed in species of *Gracilaria*. For example, if grazers remove the tip of only some of many fourth order ramets in a given clone, the grazed ramets will develop a more branched morphology than the non-grazed ramets. Our experimental studies with *G. chilensis* have failed to find evidence of physiological integration (as defined in van Groenendael & de Kroon, 1990) among high order ramets. Therefore, whatever endogenous factor might be responsible for the changes in branching pattern of the pruned ramet, it is likely to affect that part of the clone only. Since it is a physiological modification, this intra-clonal difference is likely to be lost in successive generations of cuttings from the original clone.

(b) Pathogen infections

Epi- and endophytic infections within a clone also may induce intra-clonal variations in phenotypic responses. Cell-to-cell spread, age specific variations in tissue susceptibility or physical break-up of the clone may determine that the infection be localized in one or a few ramets, causing intra-clonal phenotypic variations (Silander, 1985).

A number of studies (reviewed by Fletcher, 1995) have reported several types of epiphytes on species of *Gracilaria*. Since the settlement and germination rates of specific propagules can be selectively stimulated by various external factors, including substances released from the host (Santelices & Varela, 1993b), it is likely that many such infections are localized within a clone. Studies with other red algae have shown that host specificity may be ecologically determined (e.g. Harlin, 1973; Dickson & Waaland, 1985), may be regulated by the cell-wall composition (Craigie *et al.*, 1992) or by other structural or metabolic characters of the host (Evans *et al.*, 1978; Nonomura & West, 1981; Goff, 1982; Correa *et al.*, 1987; González & Goff, 1989; Correa & McLachlan, 1991).

(c) Genetic instability

Intra-clonal variation can also arise from several kinds of genetic changes that include somatic mutations, presence of mobile genetic elements (transposons), gene duplications as a result of errors during mitosis or intragenomic recombinations (Buss, 1985). Somatic recombination seems to be the most frequent type of change (Buss, 1985; Silander, 1985; Harper, 1985; Watkinson & White, 1986). It can occur during ramet replication modifying the entire ramet, or part of it. Somatic (mitotic) recombinations have been found to be widespread in spores of *Gracilaria* (van der Meer & Todd, 1977). These recombinations may occur in any diploid organism, but when they occur during the development of heterozygous gonimoblast tissue they may develop patches of carpospores with different genotypes. In turn, these genotypes may produce sporelings with different phenotypic attributes, such as growth potential or morphology. A similar argument applies to mitotically derived ramets of a single genet.

The frequency of mitotic recombinations in *G. chilensis* is unknown. However, in *G. tikvahiae*, van der Meer and Todd (1977) found the frequency to be high since many small, apparently independent, spots of recombinant tissue were found on the main thallus of any one plant. Further, the pattern of recombinant tissue in some of the branches strongly suggested that mitotic recombinations may occur more than once in a cell line.

(d) Sporeling coalescence

Spores of species of *Gracilaria* have the ability to grow together forming a completely coalesced mass that may later develop into a single plant (Jones, 1956; Maggs & Cheney, 1990). Among the Rhodophyta, this ability is also exhibited by members of the order Gigartinales (Rosenvinge, 1931; Chen & Taylor, 1976; Tveter & Mathieson, 1976; Rueness, 1978).

Experimental results with *Gracilaria chilensis* (Muñoz & Santelices, 1994 and ongoing research) suggest that spore coalescence may be a cause of intra-clonal variation in this species. Even though the erect shoots from few and from many coalesced spores arise

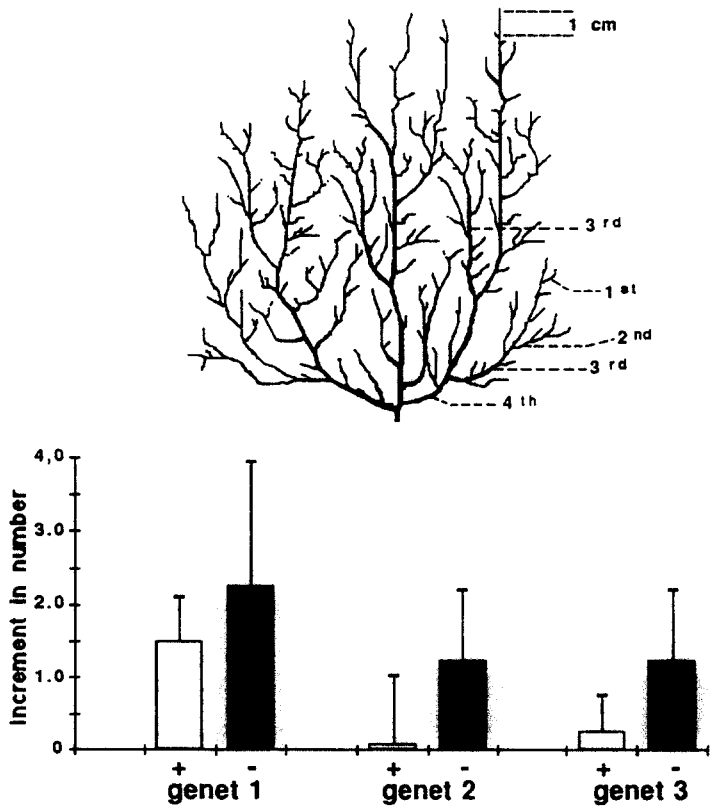

Fig. 4. Increments in the number of third order branches on fourth order branches of *Gracilaria chilensis* grown for 30 days under controlled laboratory conditions (14±2 °C; 50 μmol m^{-2} s^{-1}; 12 h of daily light). Results compare responses of 3-fourth order ramets of three different genets with (+) and without (−) the apical 1 cm. Numbers in the *Gracilaria* diagram refer to orders of branches.

more or less at the same time (Fig. 5), the total number of erect axes produced by the coalescent spore masses after 19 weeks of development is a function of the original number of spores (Fig. 6). Extreme length differences among these erect axes are found in sporeling masses originating from the coalescence of few spores while the erect axes originating from sporeling masses with large number of spores are more homogeneous in length (Fig. 7).

The more homogeneous size distribution of erect axes in large, coalescent spore masses suggest, as anticipated by Maggs and Cheney (1990), some growth regulation within the sporeling mass. However, the significant correlation found between the number of spores and the number of erect axes, also suggest the maintenance by the spores and their derivatives of their growing potential into erect axes. Perhaps the degree of early cell fusion in the coalesced sporelings does not involve all spores. Perhaps some of them divide repeatedly before some of its derivatives fuse with derivatives of other spores. If that is the case, coalesced spore masses may be a mozaic of spore derivatives. Under those circumstances, it would be expected that different ramets arising from the same holdfast and seemingly belonging to the same genet would, in practice, correspond to genetically different axes arising from tissues that originated from genetically different spore derivatives. In those cases, intra-clonal (inter-ramet) differences in morphology and performance should be common. Such intra-clonal differences are likely to express when comparing ramets of a single genet but they should not appear when comparing successive generations of cuttings from the same original ramet.

5. Implications of intra-clonal variation

As commented earlier, examples of intra-clonal variation through the spontaneous appearance of morphological or physiological variants in natural or cultivated seaweed populations have been known for some

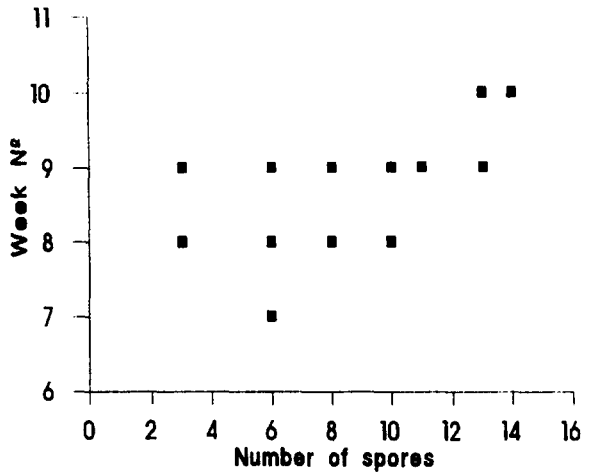

Fig. 5. Time of appearance of erect axes arising from coalescent sporeling masses of *Gracilaria chilensis*. The value of the regression equation is $y = 7.92 + 0.09x$; $r^2 = 0.19$; $p = 0.07$. Incubation methods as described in Muñoz and Santelices (1994).

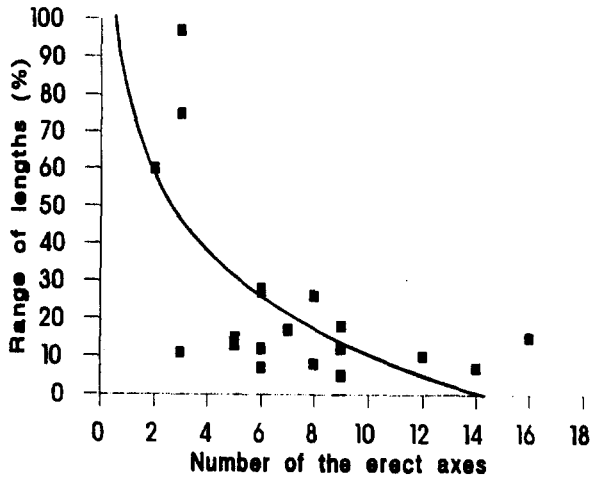

Fig. 7. Range of lengths between the longest and the shortest erect axes found in any sporeling mass of *Gracilaria chilensis* as a function of the number of erect axes. The value of the regression equation is $y = 79.59 - 68.82 \log(x)$; $r^2 = 0.44$; $p = 0.001$. Incubation and measurement methods as described in Muñoz and Santelices (1994).

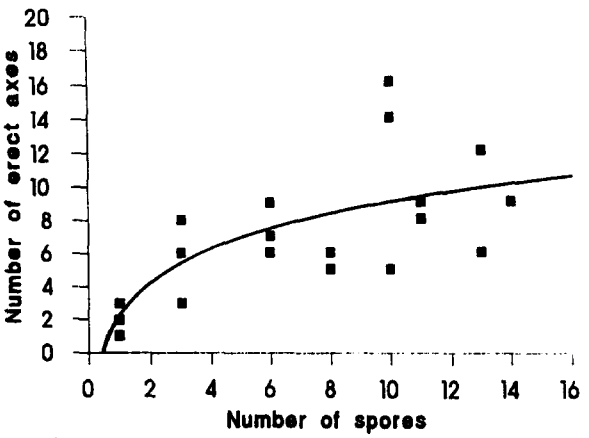

Fig. 6. Abundance of erect shoots in coalescing sporelings of *Gracilaria chilensis* as a function of the number of spores that germinated and coalesced. The value of the regression equation is $y = 2.11 + 6.86 \log(x)$; $r^2 = 0.53$; $p = 0.0001$. Incubation methods as described in Muñoz and Santelices (1994).

time. However, the demonstration of the occurrence of such variation in the self-replication units of *Gracilaria chilensis* (Santelices & Varela, 1993a) has motivated re-examination of its biological implications.

Clonal growth results in particular gene or gene combinations being repeatedly expressed, repeatedly exposed to a diversity of environments and selective forces, increasing the possibility of genet survival under at least one of such environments. In addition, phenotypic heterogeneity within genets would result in ramets of the same clone appearing dissimilar, increasing further the possibilities of population survival (Harper, 1985; Silander, 1985). This idea, generally accepted for other clonal organisms, has remained untested in seaweeds. However it may explain patterns of survival of widespread clonal seaweed species in heterogeneous environments.

Intra-clonal variation explains the origin of morphological and physiological differences among ramets of a given genet. This is most important to understand taxonomic limits, evolutionary patterns and phylogenetic relationships in clonal seaweeds.

Some kinds of clonal seaweeds (e.g. *Gracilaria*) may propagate by fragmentation of ramets capable of sinking and later functioning on their own. Other kinds of seaweeds (e.g. *Gelidium*) may propagate by erect axes arising from creeping axes that may become discontinuous due to grazing or injury. If a ramet or a new erect axis is the site of intra-clonal variation, the variability that originated at the intra-individual level is later expressed at a population level (Fig. 8). This probably explains, at least partially, the great variation on a local scale (intra-population variation) which in many of these species is considered to be responsible for most of the variation found among populations.

The significant variation and changes in performance over short periods exhibited by the clones of *Gracilaria chilensis* suggest that strain selection in this type of species should be thought of as a fairly continuous process due to the instability of the clone and the

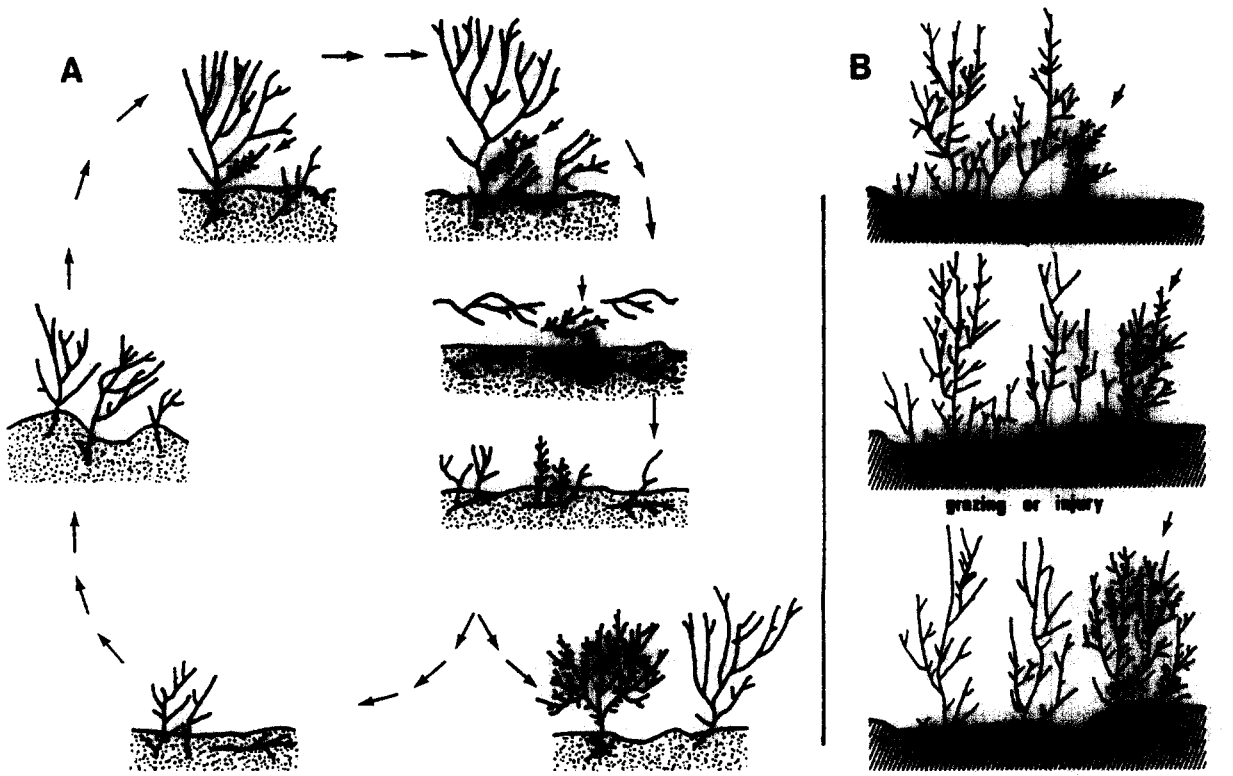

Fig. 8. Examples of how intra-clonal variation can become intra-population variation. Intraclonal variants (short arrows) may loose morphological continuity with the parental fragment. If the fragment later propagates, it will be distinguished as a genet phenotypically different from the one originating it. Figure A represents clones with vegetative propagation style similar to species of *Gracilaria*, living on sandy-muddy bottoms. Figure B represents clonal seaweeds with vegetative propagation styles similar to species of *Gelidium*, living on rocky bottoms.

unlikely long-term permanence of the selected trait in the selected strain.

Finally, the intra-clonal variation exhibited by groups of carpospores in the growth experiments, together with the evidence of genetically-based variation presented by van der Meer and Zhang (1988) suggest that carpospore production may also involve mitotic genetic recombinations and should be regarded as a stage not only for amplifying the zygote of red algae but also a point where variability among these mitotically replicated unit can be introduced.

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