Marine Biology 116, 543–552 (1993)

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Marine Biology
International Journal on Life in Oceans and Coastal Waters

Intra-clonal variation in the red seaweed *Gracilaria chilensis*

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Received: 21 January 1993 / Accepted: 25 March 1993

Abstract. The phenotypic plasticity often found in seaweed populations has been explained only from the perspective of inter-population or inter-individual differences. However, many seaweeds grow and propagate by fragmentation of genetically identical units, each with the capacity to function on its own. If significant differences in performance exist among these supposedly identical units, such differences should be expressed upon the release and growth of these units. In this study we document two such types of variation in the red seaweed *Gracilaria chilensis*. Populations of sporelings, each grown under similar culture conditions and derived from carpospores shed by the same cystocarp exhibit significant differences in growth. In this species, each cystocarp develops from a simple gametic fusion, and cystocarp fusions occur too infrequently to account for the growth differences observed among recruits. In adult thalli, branches (ramets) derived from the same thallus (genet) and grown under similar conditions exhibit significant variation in growth rates and morphology. These findings have several implications. They suggest that carpospore production is not only an example of zygote amplification but that it also could increase variability among mitotically replicated units. Intra-clonal variability followed by fragmentation and re-attachment may increase intra-population variation which, in species of *Gracilaria*, is often larger than inter-population variation. In addition, the existence of intra-clonal variability suggests that strain selection in commercially important species may require a more continuous screening of high-quality strains because of frequent genotypic or phenotypic changes in the various cultivars.

Introduction

Phenotypic plasticity is common among many seaweed species, and both inter-population and inter-individual differences have been studied. Thus, the literature contains many examples of differences among clines (e.g. Lüning 1980, Bolton et al. 1983, Sheath and Cole 1984, Rice et al. 1985), among morphological or physiological ecotypes (e.g. Durako and Dawes 1980, Espinoza and Chapman 1983, Peckol and Ramus 1985, Gerard 1988), and among mutants in wild or cultivated populations (e.g. Patwary and Van der Meer 1982, 1983a, b, c).

The assumption that most seaweeds are unitary organisms has restricted the study of intra-individual differences in performance to those specific physiological processes that assume differentiation along the thallus. While studies on polarity or apical dominance help to understand spatial or temporal processes of differentiation, they are traditionally regarded as meaningless in explaining inter-individual variation within a population. However, several types of seaweeds, including the species of *Gracilaria*, grow and propagate by self-replication of genetically identical units, and these should be considered clones (sensu Cook 1985, Jackson et al. 1985). If fragmentation of the thallus takes place, either by natural processes or by injury, each of the resulting fragments is capable of functioning on its own, of displaying differences from other parts of the same clone, and of expressing at a population level the variability originated at the intra-individual or the intra-clonal level (Fig. 1).

Data from comparative studies of clonal organisms in the animal and plant kingdoms (e.g. Bonga and Durzan 1985, Jackson et al. 1985, Harper et al. 1986), reveal phenotypic differences among ramets derived from a single genet. Physiological and developmental differences among ramets within a clone, differences among the microenvironments surrounding each ramet during growth, somatic mutations, mitotic recombinations, presence of mobile genetic elements, and pathogens differentially affecting some ramets within a clone, are some of the factors that might induce significant intra-clonal variation in phenotypic expression (Buss 1985, Harper 1985, Silander 1985, Watkinson and White 1986).

Several of the above factors are known to affect seaweeds, including various species of *Gracilaria*. Their effects as potential sources of inter-individual variation in various populations, however, have not been thoroughly

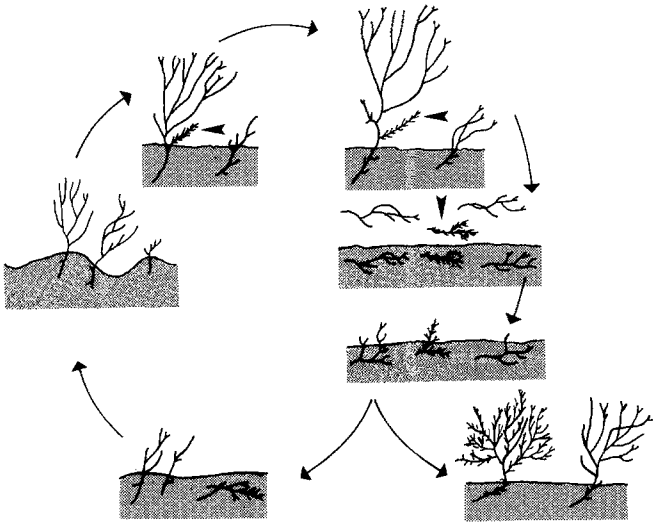

Fig. 1. *Gracilaria chilensis*. Expression at population level of variability originated at intra-clonal level. These thalli grow by self-replication of ramets and are propagated by hydrodynamic ramet fragmentation followed by sand burial and regrowth. If significant, intra-clonal differences arise in a given ramet, represented here by changes in branching pattern (arrowheads); such variation may eventually reach population level

studied. For example, physiological and developmental differences among ramets within a clone would be expected, at least in *G. chilensis*, because thallus fragments exhibit polarity (Collantes et al. 1990). Mitotic recombinations have been documented in *G. tikvahiae* (Van der Meer and Todd 1977), and the involvement of transposable genetic elements is suspected in the occurrence of unstable mutations in ramets of three species of *Gracilaria* (Van der Meer and Zhang 1988). Also, intra-clonal variations spontaneously originating and involving morphological and physiological responses, have been described for *G. secundata* (Lignell and Pedersen 1989). Further, there is experimental evidence that different types of pathogens differentially affect thallus parts (e.g. Correa et al. 1988, Müller et al. 1990, Correa and McLachlan 1991, Müller 1991 a, b, 1992), probably affecting the morphological or physiological responses of the infected ramets.

In this study we report two previously undescribed types of variation in *Gracilaria chilensis* which are interpreted as intra-clonal variation. One involves differences in elongation rates among germlings grown from carpospores of the same cystocarp. In this species, carpospores are considered as mitotically-replicated dispersal units, originating from a single gametic fusion (Fredericq and Hommersand 1989). Variability also occurs in the branching pattern and growth rates among branches (ramets) derived from mitotic divisions of a single genet. Documentation of intra-clonal variation should lead to a better understanding of patterns of phenotypic variation and evolutionary processes in *Gracilaria* species, as well as suggesting ways of improving the strain-selection practices that are currently used for these economically important species.

Materials and methods

Variation patterns among spores

Carpospores were gathered from a population of fertile, attached gametophytes of *Gracilaria chilensis* collected at low intertidal levels in Niebla (39°48′S; 73°24′W), southern Chile. They were air-shipped in temperature-controlled containers to the laboratory in Santiago, where the thalli were thoroughly washed in sterile seawater. Individual cystocarps from several plants were left in 25 ml of culture medium (SWM-3-modified, McLachlan 1973), in darkness, until spores were liberated (normally overnight). In the first experiment (24 April 1990; "April experiment"), a dissecting microscope and pipettes were used to divide each spore mass into several groups, which were transferred to individual dishes (60 × 15 mm) for further incubation. Each replicate of a similar cystocarp was designated R_1, R_2, R_3 or R_4. Similar procedures was used in the second experiment (5 December 1990; "December experiment"), except that each cystocarp was allowed to sporulate twice. After the first spore release, replicate spore masses were allocated among individual dishes for further incubation and labelled as replicates "A"s (A_1, A_2, A_3, etc.). Subsequently, each cystocarp was carefully but throughly washed to remove any attached spores and left overnight in a new dish with SWM-3 culture-medium. The spore masses released at the second shedding were identified as replicates B's. All spore masses were then incubated under controlled conditions of temperature (14 °C), photoperiod (12 h light:12 h dark) and photon-flux density (40 to 45 μmol m^{-2} s^{-1}) provided by cool-white fluorescent lights (40 W). The culture medium was changed every 5 d, and gentle air-bubbling was applied to all replicate dishes after the first week of culture.

The April and December experiments differed in two aspects. The April experiment was aimed mainly at comparing inter-plant, and inter- and intra-cystocarp variation. Therefore, 4 individual plants and up to 11 cystocarps were used. The December experiment was intended to test intra- versus inter-cystocarp variation. Therefore, emphasis was placed on increasing the number of replicated spore masses studied from each cystocarp, as well as the number of replicated cystocarps from a single plant (2 individual plants used).

The April and December experiments also differed in spore density per container. Even though in both experiments care was taken to work with isolated, individual spores only, in the April experiment no effort was made to standardize the number of spores per surface unit, and so their abundance ranged from 8 to 51 attached thalli per mm^2 bottom surface. In the December experiment, the abundance ranged only from 1 to 16 spores per mm^2 bottom surface. In the two experiments, thallus elongation was independent of germling density, since the slope of the respective regression value was not significantly different from zero (April experiment: $y = -2.789\,x + 670.85$; $r^2 = 0.024$; df 21; $p = 0.493$; December experiment: $y = -0.128\,x + 202.075$; $r^2 = 0.05$; $df = 21$; $p = 0.9539$).

After a month of incubation, the diameter of the basal area of each germling and its total length were measured in each replicate dish with a compound (inverted) microscope. Statistically significant differences in elongation rates between plants, cystocarps and replicated spore masses were tested using a three-factor ANOVA followed by Fisher's protected least-significant difference (PLSD) *a posteriori* test.

Fusions among fertilized carpogonia and growing cystocarps

Ten samples of fertile gametophytes gathered in Niebla were fixed and maintained in a 5 to 10% formaldehyde solution in seawater. Sections of fertile branches were obtained with a freezing microtome and were stained either with aniline blue or with aceto-iron-haematoxylin-chloral hydrate. Mounting was done as described by Hommersand and Fredericq (1988).

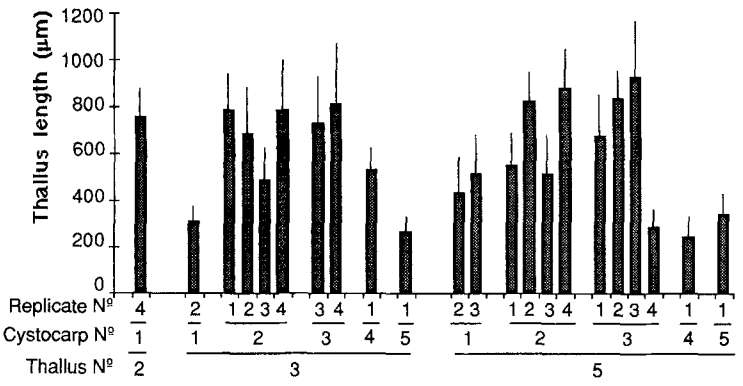

Fig. 2. *Gracilaria chilensis*. Differences in length among populations of germlings derived from cystocarps in various thalli ("April experiment"). Bars show standard deviations

Temporal persistence of differences in elongation rates

Thirty days after spore germination, the germlings of the April experiment were classified into two types. Eleven dishes showing the shorter germling populations were labelled as "slow growers" whereas 13 dishes containing taller germlings were labelled as "fast growers". Then, all but five representative germlings were removed from each dish and the dishes were ranked according to the average length of their germling population and returned to the controlled conditions described above. Length and number of branches of germlings were determined monthly for the subsequent 6 mo, and the last measurement was used to rank the dishes again.

The degree of association between first and last rankings over all dishes was tested using Spearman's rank-correlation coefficient. Kendall coefficient-of-concordance was used to test for the persistence in time of the size-ranking assigned to each dish at the beginning of the experiment (Siegel and Castellan 1988).

Variation among ramets

Elongated ($\simeq 50$ cm long) thalli from Maullin in southern Chile (41°36'S; 73°36'W) were initially collected to assess growth differences among ramets derived from a single genet. After 15 d acclimation in the laboratory, five thalli (= genets) were randomly selected. Twenty four 60 mm-long branches were cut from each clone; these were tagged, their branches were counted, and they were then photographed under water to avoid dehydration, and wet-weighed (blotted weight) in a Sartorious balance. Three ramets of each thallus were placed inside each of eight replicate 1000 cm^3 beakers filled with 900 cm^3 SWM-3 culture-medium. Because five clones were used, each beaker contained a total of 15 ramets, which were kept at the bottom by attachment to glass slides. The order of attachment of each ramet was random. Ramets were incubated under controlled conditions of temperature (14 to 16 °C), photoperiod (12 h light : 12 h dark), and photon-flux density (35 to 40 µmol m^{-2} s^{-1}). The culture medium was changed every 6 d. Length, blotted weight and number of branchlets of each ramet were recorded after 30 d. Inter-clone and inter-ramet differences were tested by ANOVA (random-block model) followed by *a posteriori* tests (Fisher PLSD test). Departure of individual ramets from the expected growth value was tested for deviations which were suspiciously large in a population (Snedecor and Cochrane 1967).

A second experiment, designed to assess significant intra-clonal differences in ramet growth, was applied to two morphologically different strains of *Gracilaria chilensis* collected in Maullín. One strain was thin and highly branched, the other thick and sparsely branched. Seven ramets from each clone, each consisting of a single, branched or unbranched axis, were selected, cleaned and incubated for acclimation for 1 mo in 1000 cm^3 beakers under the controlled conditions described earlier. At the beginning of the experiment, each thallus was cut half-way through its length; each half was then measured and photographed, its branches were counted while the thallus was under water and it was finally blot-weighed and incubated for 1 mo. Measurements were repeated after 30 d. Inter-strain and inter-genet differences in growth rates were tested using factorial ANOVA followed by Fisher's PLSD a posteriori test. Intra-clonal differences were assessed with *t*-tests for paired comparisons (Snedecor and Cochrane 1967). If temporal or spatial processes of differentiation were to induce differences among the two halves of each *G. chilensis* thallus, it would be expected that these would be consistent among all replicates (e.g. apical halves growing faster than bottom halves). If differences were not consistent, then other sources of variation could be important.

Table 1. *Gracilaria chilensis*. Differences in thallus length among populations of sporelings derived from different cystocarps in given plant. C: cystocarp; NS: non-significant difference; *: significant difference ($p < 0.05$)

	Plant No. 3				Plant No. 5			
	C2	C2	C4	C5	C2	C3	C4	C5
C1	*	*	*	NS	*	NS	*	*
C2	–	*	*	*	–	*	*	*
C3		–	*	*		–	*	*
C4			–	*			–	NS

Results

Variation in elongation rates among spore groups

In the April experiment, thallus lengths of *Gracilaria chilensis* measured 1 mo after germination showed significant inter-plant, as well as inter- and intra-cystocarp variation (Fig. 2). Significant differences in length among sporelings derived from cystocarps of any one plant were frequent (Table 1). However, intracystocarp differences were also frequent. In this experiment, 15 out of 20 intra-cystocarp comparisons yielded significant differences (Table 2).

Although with lower frequency, significant differences in length in inter- and in intra-cystocarp comparisons also occurred in the December experiment (Fig. 3, Table 3).

Table 2. *Gracilaria chilensis.* Differences in thallus length among populations of sporelings derived from various plants and cystocarps in April experiment. P: plant; C: cystocarp; R: replicate for given cystocarp; NS: non-significant difference; *: significant difference at $p \leq 0.05$ (Fisher PLSD test). Parentheses indicate comparisons among populations of sporelings grown from spores from same cystocarp

	P2 C1 R4	P3 C1 R2	P3 C2 R1	P3 C2 R2	P3 C2 R3	P3 C2 R4	P3 C3 R3	P3 C3 R4	P3 C4 R1	P3 C5 R1	P5 C1 R2	P5 C1 R3	P5 C2 R1	P5 C2 R2	P5 C2 R3	P5 C2 R4	P5 C3 R1	P5 C3 R2	P5 C3 R3	P5 C3 R4	P5 C4 R1	P5 C5 R1
P2 C1 R4		*	NS	NS	*	NS	NS	*	*	*	*	*	*	*	*	*	NS	*	*	*	*	*
P3 C1 R2			*	*	*	*	*	*	*	NS	*	*	*	*	*	*	*	*	*	NS	*	NS
P3 C2 R1				(*)	(*)	(NS)	NS	NS	*	*	*	*	*	NS	*	*	*	NS	*	*	*	*
P3 C2 R2					(*)	(*)	NS	*	*	*	*	*	*	*	*	*	NS	*	*	*	*	*
P3 C2 R3						(*)	*	*	NS	*	NS	*	NS	*	NS	*	*	*	*	*	*	*
P3 C2 R4							NS	NS	*	*	*	*	*	NS	*	*	*	NS	*	*	*	*
P3 C3 R3								(NS)	*	*	*	*	*	*	*	*	NS	*	*	*	*	*
P3 C3 R4									*	*	*	*	*	NS	*	*	*	NS	*	*	*	*
P3 C4 R1										*	NS	*	NS	*	NS	*	*	*	*	*	*	*
P3 C5 R1											*	*	*	*	*	*	*	*	*	NS	NS	NS
P5 C1 R2												(*)	NS	*	NS	*	*	*	*	*	*	*
P5 C1 R3													*	*	*	*	*	*	*	*	*	*
P5 C2 R1														(*)	(NS)	(*)	*	*	*	*	*	*
P5 C2 R2															(*)	(NS)	*	NS	NS	*	*	*
P5 C2 R3																*	*	*	*	*	*	*
P5 C2 R4																	*	NS	NS	*	*	*
P5 C3 R1																		(*)	(*)	(*)	*	*
P5 C3 R2																			(NS)	(*)	*	*
P5 C3 R3																				(*)	*	*
P5 C3 R4																					NS	NS
P5 C4 R1																						*

Table 3. *Gracilaria chilensis.* Differences in thallus length among populations of sporelings derived from various plants and cystocarps in December experiment. A or B = replicate for given cystocarp. Further details as in legend to Table 2

	P1 C1 A1	P1 C1 B1	P1 C1 B2	P1 C2 A1	P1 C2 A2	P1 C2 A3	P1 C2 B1	P1 C2 B2	P1 C3 A1	P1 C3 A2	P1 C3 B1	P1 C4 A1	P1 C5 A1	P1 C5 A2	P2 C1 B1	P2 C1 B2	P2 C2 A1
P1 C1 A1		(NS)	(NS)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P1 C1 B1			(NS)	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
P1 C2 B2				NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
P1 C2 A1					(NS)	(NS)	(NS)	(NS)	NS	NS	NS	NS	*	NS	NS	NS	NS
P1 C2 A2						(NS)	(NS)	(NS)	NS	NS	NS	NS	*	NS	NS	NS	NS
P1 C2 A3							(NS)	(NS)	NS	NS	NS	NS	*	NS	NS	NS	NS
P1 C2 B1								(NS)	NS	NS	NS	NS	*	NS	NS	NS	NS
P1 C2 B2									NS	NS	NS	NS	NS	NS	NS	NS	NS
P1 C3 A1										(NS)	(NS)	NS	NS	NS	NS	NS	NS
P1 C3 A2											(NS)	NS	*	NS	NS	NS	NS
P1 C3 B1												NS	NS	*	NS	NS	NS
P1 C4 A1													*	NS	NS	NS	NS
P1 C5 A1														(*)	NS	NS	NS
P1 C5 A2															NS	NS	NS
P2 C1 B1																(NS)	NS
P2 C1 B2																	NS

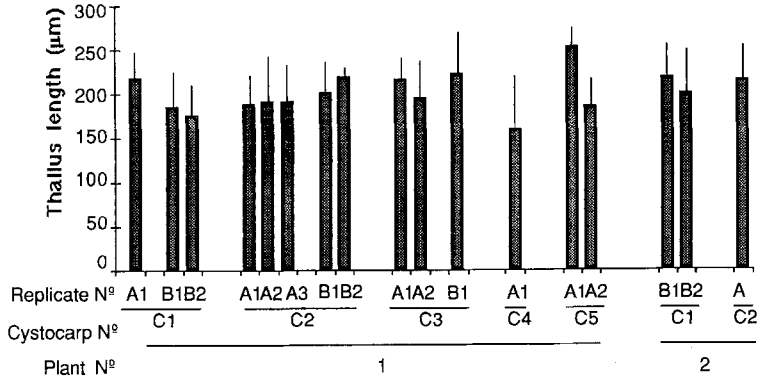

Fig. 3. *Gracilaria chilensis.* Differences in length among populations of germlings derived from cystocarps in two different thalli (Plants 1 and 2; "December experiment"). Bars show standard deviations

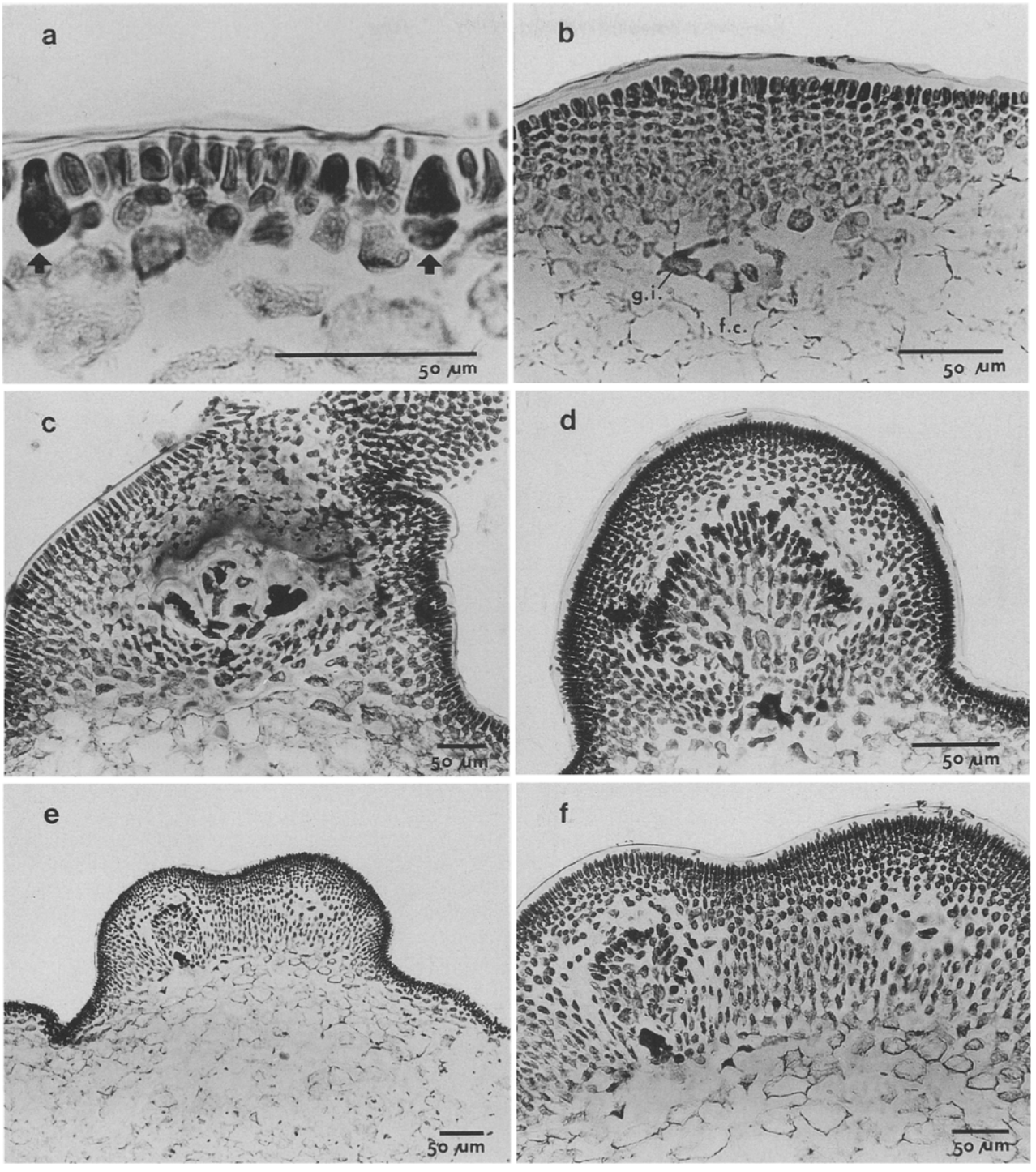

Fig. 4. *Gracilaria chilensis*. Developmental stages in cystocarp formation. (a) Unfertilized carpogonia (arrowed); (b) developing cystocarps with fusion cell (f.c.) and gonimoblast initials (g.i.) (c) more-advanced stages of development; (d) typical cystocarp with large fusion cell and rows of carpospores; (e) contiguous cystocarps that are coalescing during development; (f) enlargement of the two coalescing cystocarps

Fusions among fertilized carpogonia and growing cystocarps

The distance between unfertilized carpogonia along fertile thalli varied from 0.1 to 0.6 mm ($x=0.3$ mm, SD ± 0.2, $n=30$, Fig. 4a). The distance between fertilized, developing carpogonia, however, was 0.5 to 7.0 mm ($x=3.4$ mm, SD ± 0.8, $n=30$, Fig. 4b). Examination of 70 fertilized carpogonia yielded no evidence of carpogonial fusion during early development. In all cases, the fusion cell was observed to remain independent, originating gonimoblast lobes and later carposporangial chains (Fig. 4c, d).

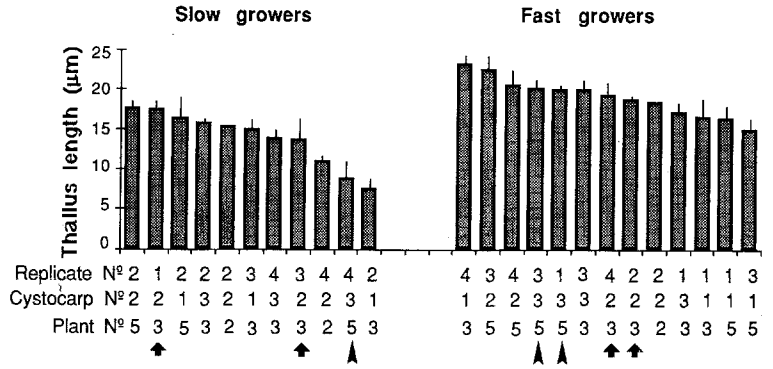

Fig. 5. *Gracilaria chilensis.* Average thallus length of 24 populations of germlings. One month after germination, individuals were sorted into slow and fast growers and then incubated for 6 mo under controlled laboratory conditions. Arrows and arrowheads indicate examples of populations of germlings derived from same cystocarp. Bars show standard deviations

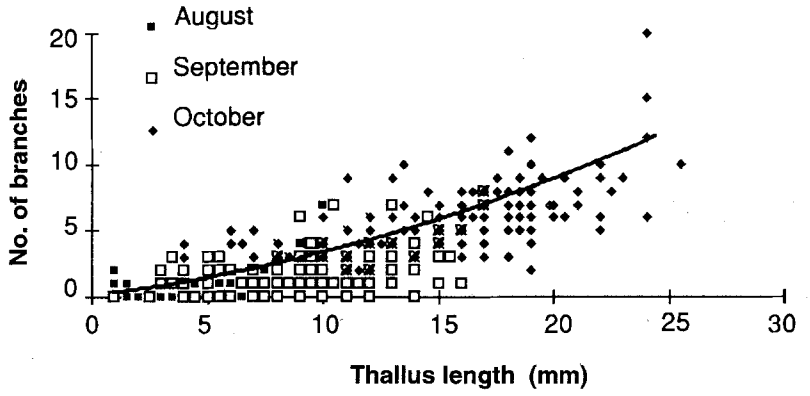

Fig. 6. *Gracilaria chilensis.* Branching density as a function of thallus length. Regression equation values: August $= y = 0.9 - 0.513x + 0.82x^2$, $r^2 = 0.672$, $p < 0.0001$, $n = 152$; September $= y = 4.287 - 0.634x + 0.044x^2$, $r^2 = 0.63$, $p < 0.0001$, $n = 147$; October $= y = 5.469 - 0.355x + 0.023x^2$, $r^2 = 0.40$, $p < 0.001$, $n = 145$; August–October $= y = 0.065 + 0.193x + 0.01x^2$, $r^2 = 0.73$, $p < 0.001$, $n = 445$

Proliferation of fertile and pericarp tissue during cystocarpic development may lead to cystocarp fusion (Fig. 4e, f) in *Gracilaria chilensis*. However, the frequency of such fusions in the materials examined was only 3% ($n = 70$).

Persistence of differences in thallus elongation rates among spore groups

On average, the populations characterized as slow growers in May (Fig. 5) elongated, between May and October, at about two-thirds the elongation rates exhibited by the populations characterized as fast growers (x: 13.9, SE = 3.4 vs x: 19.1, SE = 2.4). Further, 3 populations classified as slow growers exhibited the shortest thalli 6 mo later, whereas 7 of the 11 populations classified as fast growers exhibited the longest juveniles. Spearman's rank-correlation coefficient indicated significant association [$t(r_s) = 8.589$, $df = 29$, $p = 0.001$] between the size-rankings obtained 1 and 7 mo after germination. In addition, Kendall's coefficient of concordance of monthly measurements of thallus length indicated that the size rank assigned to each group of embryos was persistent through time ($\chi^2 = 59.70$, $df = 30$, $p = 0.001$).

Significant differences in length occurred among juveniles derived from different plants and different cystocarps within a plant. However, some of these differences arose also from spore populations gathered from the same cystocarp. For example Replicates 1 and 3 of Cystocarp 2 of Plant 3 (arrows in "slow growers" in Fig. 5) were significantly shorter than Replicates 2 and 4 of the same cystocarp (arrow in "fast growers" in Fig. 5). Some of the replicates derived from Cystocarp 3 of Plant 5 (arrowheads in Fig. 5) also displayed such differences.

In the culture experiments, branching started at lengths of 1 to 2 mm and continued in proportion to thallus length (Fig. 6). Therefore, at this stage, reduced elongation rates were not compensated by increased branching; rather, faster elongation implied increased branching and, therefore, increased biomass production.

Variation among ramets of a single genet

On average, weight increments of ramets derived from Thallus No. 5 was 30 and 45% less than the weight increment shown by any of the other four plants (Fig. 7). A multiple-comparison test indicates that such difference was statistically significant [Student-Newman-Keuls (SNK) test, $p \leq 0.05$]. However, no significant differences were found among the three series of 8 ramets in any of the five plants studied (Fig. 7). Each of the three series in each plant displayed the total range of variation exhibited by the individual thallus. Because the replication in each series was adequate, no significant differences were detected, despite the obvious intra-clonal growth differences exhibited by all clones. The plants with faster growth generally were those with larger ranges of variation.

Statistical testing of a deviation that looked suspiciously large seemed to be an adequate method of calcu-

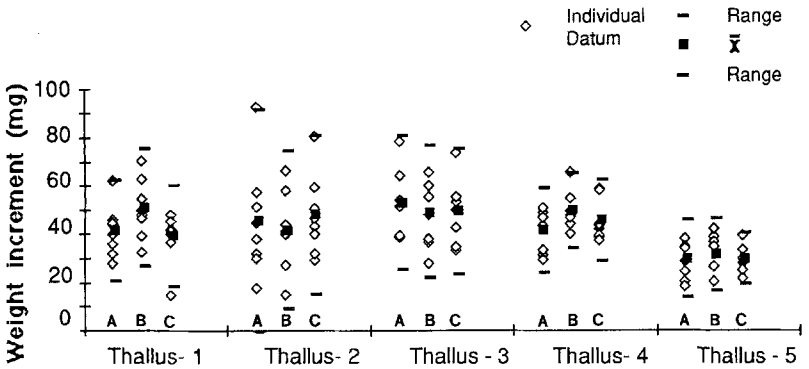

Fig. 7. *Gracilaria chilensis.* Growth increments of 24 replicate ramets, grouped in 3 blocks (A, B and C), derived from 1 of 5 different thalli. Ranges indicate 2 standard deviations

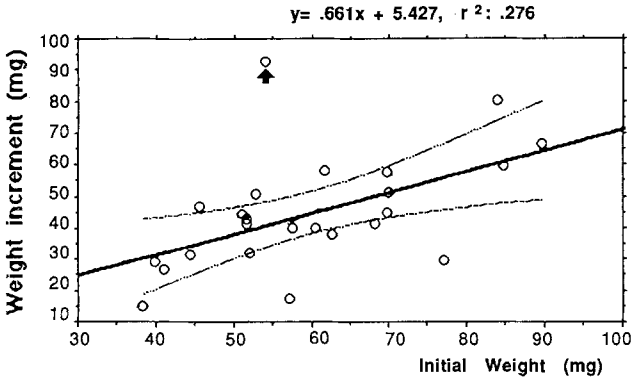

Fig. 8. *Gracilaria chilensis.* Growth increments of 24 replicate ramets from single genet as function of initial weight. Note data dispersal. Arrow indicates anomalously large deviation. Curves are confidence limits

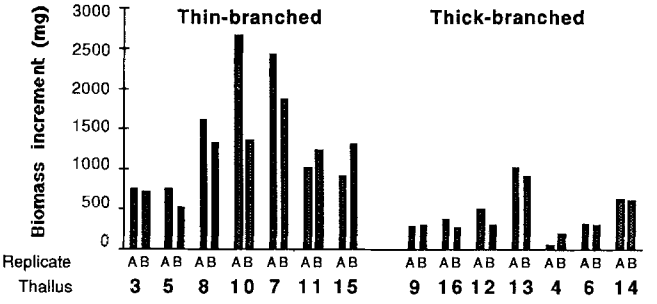

Fig. 9. *Gracilaria chilensis.* Comparison of growth increments of two morphologically different strains. Each number corresponds to one thallus. A, B: ramets derived from apical (A) or basal (B) portions of genet

lating departures of individual ramets from the population mean value. The biomass increments after 30 d of growth of the 24 ramets gathered from Plant 2 are shown in Fig. 8. In this species, production increases as a function of initial biomass. The relation is significant ($y=0.728x-0.803$, $r^2=0.482$; $df=22$, $p=0.02$) if the larger deviation is omitted (arrowed value in Fig. 8). However, the regression line is rendered non-significant ($y=0.661x+5.427$; $r^2=0.276$, $df=23$; $p=0.08$) if that value is reinstated. This extreme data point shows a significant departure ($p\leq 0.001$) from the confidence limits determined for the regression equation (92.5 mg vs 47.09 ± 6.81).

The above value was the most extreme departure found among the 120 ramets studied, but more modest, intra-clonal variations in growth responses were common. In this experiment, the growth responses in 10 of the 24 ramets derived from Plant 2 fell outside the regression confidence limits (Fig. 8). A similar phenomenon occurred with 3 of the 4 other plants. In the fourth plant, biomass increase as a linear function of initial biomass was not statistically significant ($y=0.086x+36.43$; $r^2=0.012$; $p=0.62$). However, 2 of the 24 ramets measured exhibited significant departure from the expected value.

The growth-rate values obtained in the paired experiment exhibited growth differences between strains, between plants within a given strain, and between parts of a single thallus (Fig. 9; Table 4). The "thin-branched" strain grew significantly more than the "thick-unbranched" strain ($F=13.099$; df 1; $p\leq 0.01$). Inter-genet (inter-thallus) differences were generally significant when comparing the faster growing plants of the "thin-branched" strain (e.g. P_8, P_7, P_{10}) with the slower growing plants (e.g. P_4, P_6, P_{11}, P_{12}, P_{14}, P_{16}) of the "thick-unbranched" strain. However, intra-strain differences also occurred: P_3 and P_5 of the "thin-branched" strain grew significantly less than P_7 or P_{10} of the same strain. In fact, on a growth-rate basis, P_3 and P_5 could not be distinguished from some of the genets of the "thick-unbranched", slow-growing strains. Similar to the first experiment, where plants with faster growth rates displayed a higher variance in growth rates among ramets, the faster growing strains in the present experiment also exhibited a wider range of variation among individual genets.

Growth comparisons between paired thallus portions of each genet revealed significantly different (Student's $t=2.806$, $df=27$, $p\leq 0.005$) growth rates between the two portions. The apical portion grew faster in some plants (e.g. P_5, P_8, P_{10}, P_7, P_{13}), but not in others (e.g. P_{11}, P_{15}). Because a significant part of the biomass increase in these strains was accounted for by increments in the number of branches ($y=4.795x+269.53$, $r^2=0.542$, $p=0.0055$),

Table 4. *Gracilaria chilensis*. Significance of differences in weight increments among thalli of two morphologically different strains. Clones No. 3, 5, 7, 8, 10, 11 and 15 are from thin-branched strain. Clones 4, 6, 9, 12, 13, 14 and 16 from thick-unbranched strain. Significance levels as in legend to Table 1

	Clone No.												
	4	5	6	7	8	9	10	11	12	13	14	15	16
3	NS	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS
4		NS	NS	*	*	NS	*	NS	NS	NS	NS	NS	NS
5			NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS
6				*	*	NS	*	NS	NS	NS	NS	NS	NS
7					NS	*	NS	NS	*	*	*	*	*
8						*	NS	NS	*	NS	NS	NS	*
9							*	NS	NS	NS	NS	NS	NS
10								NS	*	NS	*	*	*
11									NS	NS	NS	NS	NS
12										NS	NS	NS	NS
13											NS	NS	NS
14												NS	NS
15													NS
16													

these differences indicate that different portions of the same genet branched (and increased in weight) at different rates under similar culture conditions.

Discussion

Intraclonal variation was frequent in the two replication systems studied in *Gracilaria chilensis*. Germlings grown from spores of the same cystocarp elongated and branched at significantly different rates, and the same was found when various ramets derived from the same genet were incubated under similar growth conditions. These differences have not been described previously in seaweeds, since the replication units have been presumed to be genetically and phenotypically identical. Since new erect thalli can originate either from individual spores or from thallus fragments, intraclonal variability can eventually be expressed at a population level, significantly increasing the amounts of intrapopulation variation.

Growth differences among germlings from spore groups derived from a single cystocarp may occur if two or more zygotes (= fusion cells) fuse during development, or if cystocarps fuse during development. No evidence of zygote fusion was found in this study nor has it been reported in other fertilization studies on the genus *Gracilaria* (see Fredericq and Hommersand 1989 and references therein). Further, the distance between carpogonia increased after fertilization. This may result from cell proliferation during cystocarp development or from inhibition of unfertilized carpogonia by their fertilized neighbours. Whatever the reason, increased distance diminishes the likehood of zygote or cystocarp fusions. The observed frequency of intra-cystocarp differences in sporeling growth rates appears to be much higher than the observed rate of zygote or cystocarp fusions, and cannot be explained on the basis of cystocarp fusions.

The growth differences observed among ramets of the same genet also have no obvious explanation. All ramets in a given experiment were handled in the same way and care was taken not to affect the growing tips, which in other species of *Gracilaria* have been found to be sensitive to desiccation (Hodgson 1984). Special efforts were made to provide similar growing conditions to all replicates in order to reduce differences in the microenvironment around the ramets. Physiological or developmental differences among ramets were 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 branch, and by using ramets containing a similar number of branches. However despite our efforts, the observed differences may have reflected undetected microenvironmental differences in the culture conditions. Additional, more precise, studies will be needed to evaluate this possibility, which seems unlikely given the similarity of conditions used and the magnitude of the observed differences.

The present study provided no clear explanation of the significant intra-clonal differences among ramets of *Gracilaria chilensis*, but a number of factors could be involved. Besides somatic or microenvironmental factors, which in this series of experiments were intended to be minimal, a number of genetic factors such as mutations, mitotic recombinations, and genetically unstable elements, could affect the replicability of the branching system. The differences in growth rates and branching patterns among spore groups and among ramets seem too frequent to be explained by the currently documented rates of mutations in marine algae (Russell 1985, Van der Meer 1987). Mitotic recombinations however, have been found to be widespread in species of *Gracilaria* (Van der Meer and 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 differing in phenotypic attributes, such as growth potential or morphology. A similar argument applies to mitotically derived ramets of a single genet. Mitotic recombi-

nations have been identified and proposed by Van der Meer and Todd (1977) as accounting for the occurrence of mixed reproductive phases (gametophytic spots in tetrasporophytic thalli) in *G. tikvahiae*, a situation also reported for *G. chilensis* (Prieto et al. 1991). In *G. tikvahiae*, the frequency of mitotic recombination was considered high by Van der Meer and Todd, 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 lateral branches strongly suggested that mitotic recombinations may occur more than once in a cell line.

Unstable mutations also seem to be common among species of *Gracilaria*, as Van der Meer and Zhang (1988) have documented them in *G. tikvahiae*, *G. foliifera* and *G. sjoestedti* (= *G. lemaneiformis*). Cells in the thallus of a green mutant reverted to the red type, originating flecks of red tissue. Reversions occurred during all phases of the life cycle, including vegetative ramets and developing carposporophytes. Some cystocarps that formed on green portions of the green females developed masses of reddish carpospores in addition to the green ones, and many of the tetrasporophytes derived from these cystocarps were of the red wild-type color. Many unstable mutants appeared to arise from the transposition of genetic elements (transposons), affecting the expression of a marker gene (e.g. a green allele). In wild plants with no marker genes, such as those plants used in the present experiments, the presence of these mobile elements might be expressed, for example, as modifications in growth, or by changes in the morphology of the affected embryo.

Additional experimental studies are required to determine the causes of the intra-clonal variations in growth rate and branching pattern found in carpospores and ramets of *Gracilaria chilensis*. However, the demonstration of the occurrence of such variation in the self-replication units of this species has several implications for our current understanding of its biology and utilization. One implication concerns our interpretations of adaptive traits in the life history of the Rhodophyta. Carpospore production after gamete fusion is considered to be a case of zygote amplification, the main effect of which is to increase the number of progeny resulting from a single fertilization event (Guiry 1978, Searles 1980, Hawkes 1990). Genetic variation is expected to arise as a result of meiosis during tetraspore formation. However, the variation in performance showed by groups of carpospores in our experiments, together with the evidence of genetically-based variation presented by Van der Meer and Zhang (1988), suggest that carpospore production may also involve genetic recombinations and should be regarded as a mechanism increasing variability among these mitotically replicated units.

Gracilaria spp. are often characterized as being among the most physiologically and morphologically variable of all seaweeds. Variation on a local scale (intrapopulation variation) is such that it is often considered to be responsible for most of the variation found among populations. Great phenotypic plasticity or genetic polymorphism have been suggested as potential explanations of this phenomenon (Carroll 1991). However intra-clonal variation ensuing from somatic or genetic mechanisms such as those mentioned above should be taken into account, as they yield considerable variation within populations. Therefore, understanding intra-clonal variation may be most important to the understanding of taxonomical limits, evolutionary patterns and phylogenetic relations in some of the taxonomically confused clonal seaweeds, such as the species of *Gracilaria*.

Because several species of *Gracilaria* are economically important (see Santelices and Doty 1989 for a review), strain selection of economically desirable clones is being undertaken in various latitudes. The possibility of indefinite growth has naturally led to the adoption of clonal selection and vegetative propagation as the most widely employed dissemination practice of seaweed farms. It is generally assumed (Hanisak and Ryther 1984) that sterile clones can be maintained for long periods of time without changes in their genetic makeup and without the need for additional selection, and that external morphology can be used to predict the production capacity of the clone (Hanisak et al. 1988, 1990). Our findings suggest that these assumptions may be of limited validity, at least for *G. chilensis*. Our clones showed significant variation and changes in performance over short periods, which suggests that strain selection is a fairly continuous process. Intra-clonal variability, in addition, diminishes the capability of precisely predicting performance based on external morphology. Our results support the suggestion that thin-branched clones grow faster than thick-unbranched clones (Hanisak et al. 1988, 1990). However, the variability is such that individual clones of the thin-branched strain may grow as slowly as individual clones of the slow-growing strain. Therefore, the aforementioned prediction does not seem to apply to individual clones within a given strain.

The overall conclusion emerging from our studies is the need to incorporate concepts from the theory of clonal organisms into the biological knowledge of the seaweeds. The long-held assumption that their replication units are genetically and phenotypically identical is not supported by our results. Since new thalli can originate either from individual spores or from thallus fragments in many types of seaweeds, intra-clonal variability can be expressed at a population level, originating a source of variability which has thus far been but poorly studied.

Acknowledgements. We thank R. Otaíza and I. Meneses for their help with the laboratory work, and R. Westermeier for providing facilities for collecting *Gracilaria chilensis* in southern Chile. Comments on variability among spore populations made by J. Van der Meer in early stages of this study are acknowledged with gratitude. Our appreciation goes to J. Correa, J. Cancino, F. Jaksic, A. Hoffmann and M. Muñoz for comments on early drafts of this manuscript. We are grateful for the financial support of FONDECYT-Chile, Grant 803/90.

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Communicated by M. G. Hadfield, Honolulu