

SPORELING COALESCENCE AND INTRACLONAL VARIATION IN *GRACILARIA CHILENSIS* (GRACILARIALES, RHODOPHYTA)¹

Bernabé Santelices,² Juan A. Correa, Isabel Meneses,
Diego Aedo, and Daniel Varela

Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile,
Casilla 114-D, Santiago, Chile

ABSTRACT

This study evaluates the hypothesis that spore coalescence may cause intracolonial variation. Spore coalescence might allow the occurrence of unitary thalli that in fact correspond to genetically different, coalesced individuals. Plant portions simultaneously derived from these chimeric individuals may exhibit dissimilar growth responses even when incubated under similar abiotic conditions. Testing of the hypothesis included various approaches. Transmission electron microscopy observations of early stages of sporeling coalescence indicated that polysporic plantlets were formed by groups of spores and their derivatives. Even though adjacent cells in two different groups may fuse, these groups maintained an independent capacity to grow and form uprights. Laboratory-grown plantlets showed a significant correlation between the initial number of spores and the total number of erect axes differentiated from the sporeling. Construction and growth of bicolor individuals indicated the chimeric nature of the coalesced individuals. Coalesced, bicolor holdfasts had green and red cells, which subsequently produced green and red uprights, respectively. Individuals fronds were also chimeric, as indicated by the production of green and red branchlets from single, red uprights. The existence of mixed tissues was further substantiated by random amplified polymorphic DNA analysis. The banding pattern produced by branchlets of a unispore thallus was consistently monomorphic, whereas the patterns produced by the polysporic thallus were polymorphic. Growth rates of polysporic thalli had larger data dispersal and variation coefficients than oligosporic or monosporic thalli. Therefore, all results support the original hypothesis and suggest that coalescence might be ecologically more important than previously thought.

Key index words: bicolor individuals; *Gracilaria chilensis*; intracolonial variation; RAPD; Rhodophyta; sporeling coalescence

Experimental field and laboratory studies have shown significant intracolonial variation in *Gracilaria chilensis* Bird, McLachlan et Oliveira. Plant portions simultaneously derived from the same clone or successive generations from the same cut may exhibit dissimilar growth responses when incubated under similar abiotic conditions (Santelices 1992, Santelices and Varela 1993).

Three kinds of factors may cause intracolonial variation: 1) physiological and developmental differences among ramets derived from a single genet, 2) pathogens differentially affecting some ramets within a clone, and 3) genetic changes in a given ramet. These factors are recognized as the most important, causing phenotypic variation among ramets derived from the same genet in organisms as diverse as Bryozoa, land plants, and seaweeds (Buss 1985, Harper 1985, Watkinson and White 1986, Santelices 1992). A fourth factor, spore coalescence, has been postulated as exclusive to seaweeds (Santelices et al. 1995) and constitutes the main focus of this study.

Spores of the orders Ahnfeltiales, Corallinales, Gigartinales, and Gracilariales (Rhodophyta) have the ability to form basal crusts that may grow together, forming a completely coalesced mass, which subsequently develops into a single plant (for specific taxa, see Maggs and Cheney 1990). The coalesced mass exhibits vertical and horizontal alignment of cells, cuticular continuity, and formation of secondary pit plugs between cells of confluent individual spores.

For *Gracilaria*, successful spore coalescence has been achieved between spores of similar or different thalli (e.g. *G. verrucosa* Jones 1956, *G. chilensis* Muñoz and Santelices 1994), or similar or different karyological phases, and even between spores of different species (*G. tikvahiae* × *G. chilensis* Maggs and Cheney 1990). Therefore, spore coalescence might allow the occurrence of unitary thalli that in fact correspond to genetically different, coalesced individuals. It would be expected that axes of these chimeric individuals may exhibit intracolonial variation in growth or performance.

Experimental data in the literature partially support this idea. Cheney (in Maggs and Cheney 1990) obtained bicolored (i.e. red and green) discoid masses of cells starting from green (mutant) and red (wild-type) spores. Subsequently, both cell types produced uprights that displayed a pigmentation identical to their parental cells, supporting the notion that genetically different fronds could come from a single, coalescing holdfast. However, although red and green fronds were produced, individual chimeric fronds were not observed, suggesting that spore coalescence could explain intracolonial variation among primary axes only and not among branchlets of the same axis.

In this study, we evaluate whether or not spore

¹ Received 5 September 1995. Accepted 19 December 1995.

² Author for reprint requests.

coalescence could be regarded as a factor causing intracolonial variation in *Gracilaria chilensis*. Using transmission electron microscopy (TEM), we first examined early stages of development to measure the extension of the coalescence and fusion processes. Combining spores from similar and different cystocarps, we constructed uni- and polycystocarpic plantlets with initially different numbers of spores and studied sporeling survival, growth pattern, and external morphology as a function of the initial number of spores. Combining green and red carpospores, we obtained bicolor individuals and studied the expression of the chimeric tissues in fully developed plants. Two plants (one unisporic and the other polysporic), which survived the experimental period, allowed us to make an exploratory analysis of the genetic variability that could be induced by spore coalescence. Preliminary DNA comparisons were made using the random amplified polymorphic DNA (RAPD) technique, which has been used, so far, to fingerprint species and populations of *Porphyra* (Dutcher and Kapraun 1994) and *Sargassum* (Ho et al. 1995) and individuals of *Gelidium* (Patwary et al. 1993, Patwary and van der Meer 1994). Using unisporic and polysporic thalli, we then evaluated whether or not the range of intracolonial variation regarding growth and performance changes as a function of the original number of spores.

MATERIALS AND METHODS

All the experiments were performed with thalli collected in Niebla, in southern Chile (39°51'S, 73°08'W). Plants were transported by air in refrigerated containers to the laboratory in Santiago. They were washed with running tap water and maintained for 24 h in filtered seawater under standard conditions of temperature ($14 \pm 2^\circ\text{C}$), irradiance ($20 \pm 10 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and photoperiod (12:12 h LD). Sporulation was induced by immersing mature cystocarps in microfiltered seawater (0.45 μm). These cystocarps were dehydrated for 24 h at room temperature prior to sporulation.

Ultrastructure of germlings originated from coalescing spores. Carpospores shed by the experimental cystocarps were inoculated into holes (1.8 mm diameter, 250–300 μm deep) drilled in glass microscope slides. The number of carpospores in each hole varied from 30 to 50, and a total of 30 cover slips was used. Individual cover slips were incubated in Petri dishes (50 \times 10 mm) containing approximately 10 mL of filtered seawater. Growth chambers were set up under the preceding conditions.

At different times, ranging from 24 h to 30 days after inoculation, spores and plantlets at different stages of development were fixed while in the depressions with 1% glutaraldehyde in 0.45- μm -filtered seawater for 24 h. Postfixation in 1% OsO₄ tetroxide for 3 h was followed by dehydration in an ethanol series. Samples were infiltrated and embedded in Spurr's resin. Semithin sections for light microscopy were stained with 1% Toluidine blue and thin sections for TEM were stained with 4% uranyl acetate and lead citrate according to Reynolds (1963). Observations were made in a Philips 300 electron microscope operated at 60 kV.

Survival, growth, and external morphology as a function of the initial number of spores. Carpospores, 1–32, were pipetted into the holes drilled in the glass microscope slides. Inoculations included carpospores shed by a single cystocarp (generating unicystocarpic plantlets) or two different (generating polycystocarpic plantlets)

cystocarps. In the latter case, the proportion of spores from each cystocarp was approximately the same. Twenty-seven cover slips contained unicystocarpic spores, and 18 contained polycystocarpic spores. All cover slips were incubated in individual glass Petri dishes (50 \times 10 mm), filled with SWM-3 culture medium (McLachlan 1973), and maintained under standard culture conditions. Growth was monitored at 3-d intervals during the first 2 weeks and weekly thereafter. To prevent diatom growth, 1 mL of a stock GeO₂ solution (6 mg·L⁻¹) was added to each liter of SWM-3. Culture medium was changed weekly.

At the tenth week of growth, cover slips with their thalli were transferred to 250-mL flasks, each containing 200 mL of SWM-3 culture medium. Gentle air bubbling was also provided. Other abiotic conditions were as already described including weekly changes of the culture medium. The experiment was started on June 1993 and was terminated 30 weeks after, when the first four experimental individuals became reproductive.

By 7–10 weeks, sporeling masses were large enough to distinguish, and the emerging erect shoots could be counted. Elongation rates and number of lateral branches produced by the erect shoots were measured every week between the tenth and the thirtieth weeks as described by Muñoz and Santelices (1994).

Data on growth patterns as functions of the original number of spores were analyzed using regression and covariance analysis (Snedecor and Cochran 1967).

Assembling and growth of bicolor individuals. The experimental procedures for this experiment were similar to those already described. However, we combined carpospores from red, wild-type thallus collected in Niebla with carpospores from a green, fertile cystocarpic thallus collected in Mejillones, near Antofagasta (23°38'S, 70°24'W) in northern Chile. Similar numbers of green and red carpospores were used to construct bicolor individuals. The initial number of spores ranged from 2 to 36, and the total number of assembled plantlets was 25. Plantlets were incubated and handled as those in the preceding experiment. However, because the main interest of producing bicolor plantlets was to study tissue distribution, quantitative measurements were replaced by recording the development of the surviving plantlets photographically. Observations of these chimeric thalli terminated after 75 weeks of growth.

RAPD. Polymerase chain reaction amplification was performed with DNA extracted from branchlets of one unisporic and one polysporic plant. Both types of vegetative thalli were 8 cm long and had been kept growing under standard culture conditions for 28 weeks prior to the extraction. The unisporic thallus had one erect axis supporting numerous lateral branches in a semi-alternate manner. The polysporic plant came from the coalescence of three spores and had three erect shoots. Shoots a and c each had three lateral branchlets, labeled a₁, a₂, and a₃ and c₁, c₂, and c₃, respectively. The third axis (shoot b) had four lateral branchlets, named b₁, b₂, b₃, and b₄. DNA from these 10 lateral branchlets as well as from 9 laterals from the unisporic thallus was extracted and analyzed. Before extraction, all branchlets were cleaned under the microscope and sonicated to avoid contaminants.

Total DNA was extracted from fresh tips, each less than 2 mm long. Individual tips were ground in 200 μL of buffer (250 mM Tris-HCl, 250 mM NaCl, 25 mM EDTA, 0.1% SDS) with a silicon carbide abrasive (carborundum) in an Eppendorf tube of 1.5 mL. Addition of 400 μL of chloroform:isoamyl alcohol (24:1) with occasional vortexing of the mixture for 10 min was followed by centrifugation at 13,000 rpm for 15 min in an Eppendorf centrifuge.

The upper aqueous phase was then transferred to a new tube, and an equal volume of 100% ethanol was added to precipitate the nucleic acids. The mixture was frozen at -70°C for 30 min, and the nucleic acids were pelleted by centrifugation for 15 min (13,000 rpm). The pellet was washed in 70% ethanol, dried, and redissolved in TE buffer (10 mM Tris HCl, pH 8.0, and 1 mM EDTA). The sample was incubated at 37°C for 20 min and centrifuged. Nucleic acids in solution were removed with a mi-

cropipette, and further purification was achieved using a Wizard Minipreps DNA purification system (Promega, Madison, Wisconsin).

Amplifications were performed in 20- μ L solutions containing Taq DNA polymerase buffer (Promega), 100 μ M of each deoxyribonucleotide triphosphate (Amersham, England), 1.0 μ M of decamer primers (Operon Technologies, California), 20 ng of template DNA, and 0.5 unit Taq DNA polymerase (Promega). The amplifications were performed in a Perkin-Elmer DNA thermal cycler model 480 programmed for 3 initial cycles of 1 min at 95°C, 1 min at 37°C, and 1 min at 72°C, followed by 40 cycles of 30 s at 94°C, 30 s at 40°C, and 1 min at 72°C, with a final run of 5 min at 72°C. Fifteen microliters of the reaction products were separated by electrophoresis through 1.0% agarose gels and stained with ethidium bromide. Agarose gels were photographed with a 2N Olympus camera with a Wratten gelatin filter 22 (KODAK) over a ultraviolet transilluminator (312 nm) using T-MAX 100 ASA film.

Variability in growth and performance. Three, 25-week-old plants were used in this experiment. One thallus (plant 2) was unisporic. One (plant 13) developed from the coalescence of three spores, and one (plant 3) developed from 11 coalescent spores. The unisporic plant had one, the 3-spore plant had two, and the 11-spore plant had three erect axes invested with lateral branchlets.

Branchlets (9–14) of similar initial weight (3–14 mg) were cut from each erect axis in each plant and incubated under the standard conditions described earlier. Experimental procedures followed the protocol described by Santelices and Varela (1993). The branchlets were tagged, and their secondary branchlets were counted. The branchlets were photographed under water to avoid dehydration and wet-weighted (blotted weight) in a Sartorius balance. Three ramets from each thallus (= genets) were placed inside each of four replicate 1-L beakers filled with 900 mL of SWM-3 culture medium. Because four genets (= individuals) were used, each beaker contained a total of 12 ramets, which were kept at the bottom by fastening to glass slides. The fastening order for each ramet was at random. The culture medium was changed every 6 d. Length and blotted weight of each ramet was recorded after 30 d. Data dispersal and coefficient of variation for each plant and each erect axis within the multi-axis thalli were compared.

RESULTS

Ultrastructure of germlings originated from coalescing spores. Once released and prior to the first mitosis, spores were naked (Fig. 1). Immediately after settling, small vesicles containing large concentrations of fibrillar material coalesced into larger ones, located at the periphery of the cell (Fig. 2). Subsequently, the large vesicles located underneath the plasmalemma began releasing the fibrillar material (Fig. 3), and plantlets with the characteristic double cell wall were present at the end of the 24-h period (Fig. 4).

Spores that settled close enough to be in contact to each other during germination showed no evidence of plasmalemmal fusion (Fig. 5). On the contrary, each of these spores produced a distinctive cell wall that separated neighbor individuals. The wall produced by each spore became the outer cell wall of individual germlings by surrounding the cells originated during spore germination (Fig. 5). During early development, some germlings shared a common external envelope that reacted strongly with Toluidine blue (Figs. 5, 6). In such cases, fusion of

the adjacent groups of cells could occur (Fig. 6). However, this was not frequent. In general, where many germlings grew close enough to establish contact, most individuals remained as discrete entities (Fig. 7).

The outer cell wall of coalescing individuals appeared as a continuum (Fig. 8), with a finely reticulate texture that clearly differed from the homogeneous aspect displayed by the immediate cell wall (Fig. 9). Secondary pit plugs were established between cells of coalescing germlings at the fusion zone (Fig. 10). There seems, however, to be no ultrastructural differences between these and the primary pit plugs.

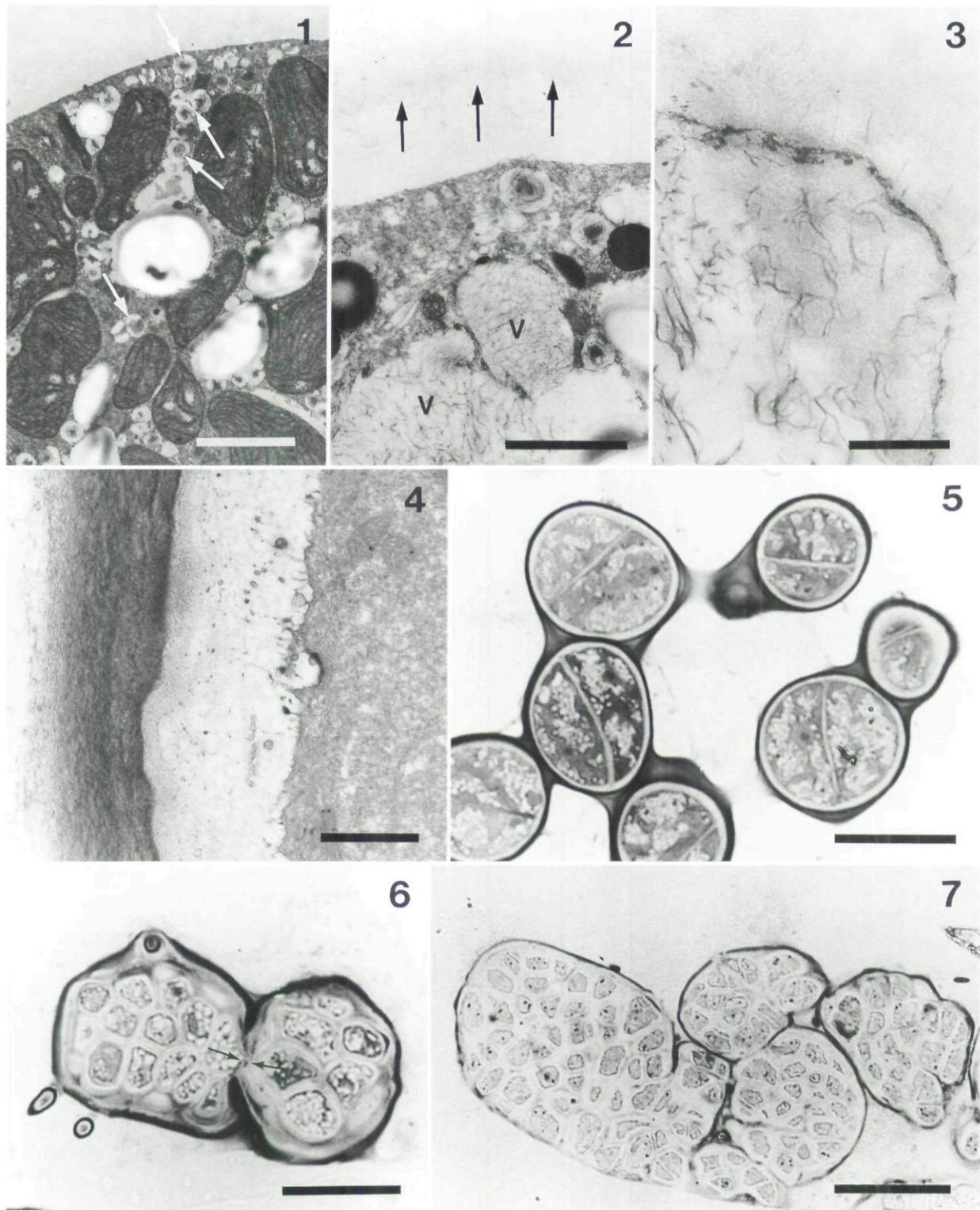
Survival, growth, and external morphology as a function of the initial number of spores. Average carpospore germination rate was $74.8 \pm 9.4\%$. Germination rate of individual spores within a coalescing mass slightly decreased with increasing spore density, but the reduction was not statistically significant for either unicystocarpic and polycystocarpic coalescing spores. Covariance analysis ($F = 2.33$, $df = 1$, $P = 0.136$) indicated an absence of significant differences in germination rates between uni- and polycystocarpic spore assemblages.

Sporeling and sporophyte development followed the pattern previously described for this species (Muñoz and Santelices 1994). Uni- and multisporic plantlets formed irregular, circular discs that gradually expanded in diameter and height. Between the seventh and tenth weeks of development, plantlets produced erect shoots, the longest of which reached about 10 mm by the fifteenth week. Four weeks later, some of these axes showed differentiation of lateral branches, and by the thirtieth week some of the longest erect axes (8–10 cm) became fertile.

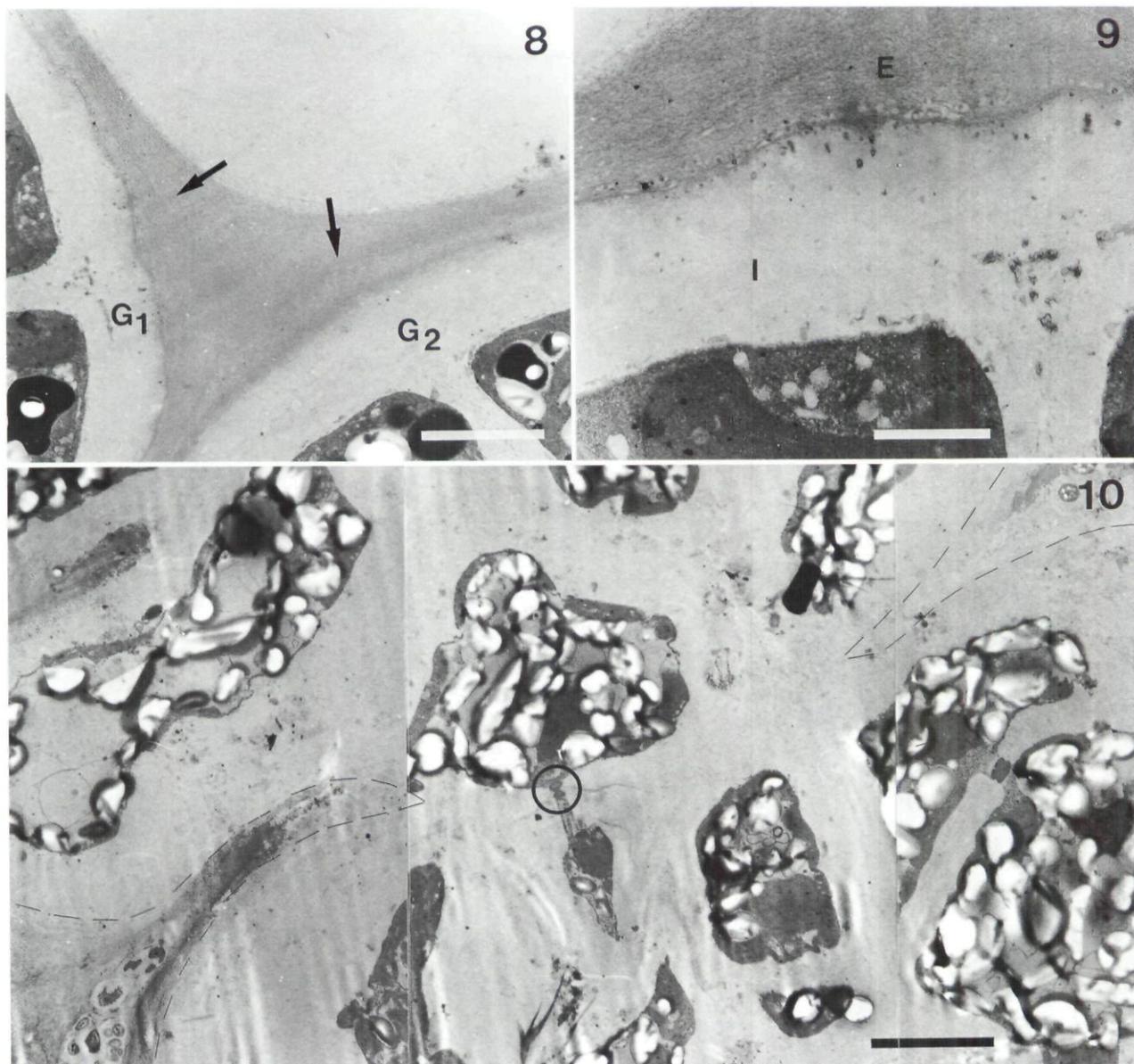
Survival rates of the plantlets during the 30 weeks of growth were essentially similar for uni- and polycystocarpic plantlets. Three of the 27 unicystocarpic and two of the 18 polycystocarpic plantlets died within the first 3 weeks. Two more plantlets of each type died in the next 2 weeks. The remaining 22 unicystocarpic and 14 polycystocarpic plantlets remained alive until the end of the experiment.

The time of appearance of erect axes ranged from the seventh to the tenth week of growth. There was a tendency for plantlets from a few coalescing spores to differentiate uprights earlier in development. However, regression values were not statistically significant for unicystocarpic nor for polycystocarpic sporeling masses.

The total number of erect axes produced by the coalescing masses of spores after 19 and 29 weeks of development was a function of the original number of spores (Fig. 11). Unicystocarpic plantlets produced more erect shoots than polycystocarpic plantlets. However, although differences were not statistically significant at the nineteenth week (covariance analysis, $F = 3.78$, $df = 1$, $P = 0.06$), they became significant by the end of the experiment (covariance analysis, $F = 4.27$, $df = 1$, $P = 0.04$). In



FIGS. 1-7. Ultrastructure of germlings originated from coalescing spores. FIG. 1. Naked spore after release. Abundant, small vesicles (arrows) containing fibrillar material appear scattered among sections of the chloroplast. Scale bar = 2 μm . FIG. 2. Settled spore with large vesicles (V) loaded with coarse fibrillar material. Extracellular material (arrows) begins accumulating outside the cell to form the cell wall. Scale bar = 1 μm . FIG. 3. Close-up of the cell surface showing the release of coarse material. Scale bar = 0.5 μm . FIG. 4. Double cell wall in an individual spore that has completed settlement. Scale bar = 1 μm . FIG. 5. The first mitotic division yields two cells separated by a low-contrast cell wall. Several of the two-cell groups have coalesced with neighboring groups through a common, external cell wall. Scale bar = 30 μm . FIG. 6. Fused plantlets 24 days after settlement. Both plantlets share a common external cell wall that tends to disappear at the intercell contact area (arrows). All other cells maintain their individuality. Scale bar = 30 μm . FIG. 7. Coalescent but unfused plantlets 24 days after settlement. Scale bar = 50 μm .



FIGS. 8–10. External cell wall and secondary pit connections between cell groups of different origins. FIG. 8. External cell wall (arrows) common to two-cell groups of different origins (G_1 and G_2) in 24-d-old plantlets. Scale bar = 3 μm . FIG. 9. Enlargement showing the immediate cell wall (I) of more homogeneous texture and the external cell wall (E) of fibrillar appearance. Scale bar = 1 μm . FIG. 10. Fusion zone between two adjacent, 24-d-old plantlets. The dotted lines indicate the limits of the coalescing plantlets. The external cell wall does not exist at the fusion zone. Secondary pit connections (circle) may occur between cells of both plantlets. Scale bar = 5 μm .

both cases, the relationship between initial number of spores and number of erect axes was logarithmic, with a decreasing number of shoots produced by plantlets formed by a larger number of spores.

The preceding numerical relationship was reflected in the external morphology of the plants. Thalli grown from one or a few spores were elongated plants with a single or few main axes bearing lateral branches. Individuals grown from many coalescent spores appeared bushy, with many short, sparsely branched erect axes arising from a discoid holdfast.

The first reproductive fronds were the longest

axes of four polysporic thalli, originated from 6, 8, 10, and 13 coalescing spores.

Assembling and growth of bicolor individuals. Germination and growth of bicolor individuals followed the normal developmental patterns although their mortality rates were much higher than those generated by coalescence of red spores alone. Only eight of the 25 initial plantlets (32%) were alive by the time erect shoots began to appear (weeks 8 and 9). Of these, only three survived long enough to exhibit initiation and development of lateral branchlets (beyond week 15). Two of these bleached by week 18,

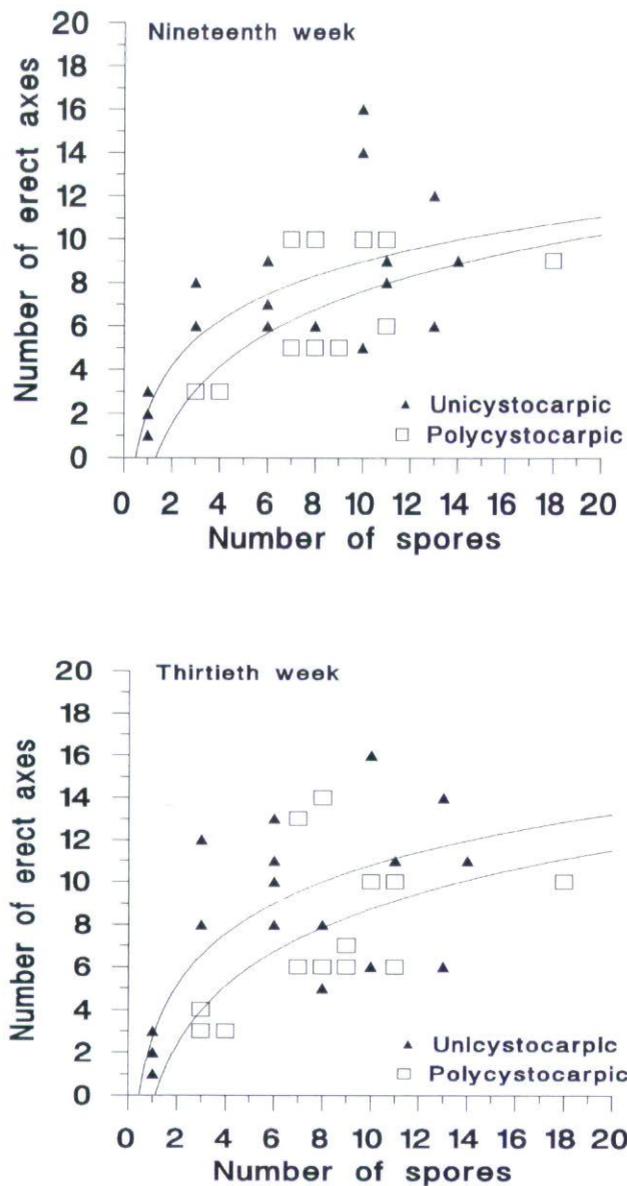


FIG. 11. Total number of uprights at two times of development as a function of the original number of spores used to construct coalescing plantlets of *Gracilaria chilensis*. Regression equation values are, for the nineteenth week, unicystocarpic plantlets (upper line): $y = 2.11 + 6.86 \log(x)$, $R^2 = 0.54$, $P = 0.0001$; polycystocarpic plantlets: $y = -1.09 + 8.69 \log(x)$, $R^2 = 0.51$, $P = 0.003$. For the thirtieth week of growth, the equation for unicystocarpic plantlets (upper line) is $y = 2.70 + 8.07 \log(x)$, $R^2 = 0.53$, $P = 0.0001$; polycystocarpic plantlets: $y = -0.41 + 9.14 \log(x)$, $R^2 = 0.39$, $P = 0.017$.

and the only surviving individual became reproductive by week 42 and remained alive and healthy for 75 weeks (Fig. 12).

All eight plantlets alive by the time of upright initiation produced green and red axes from the bicolor holdfast (Fig. 13). With time, the surface of the holdfast usually became dominated by either green or red tissue, and the color of the new shoots was that of the dominating tissue in the holdfast (Fig.

14). The plants, however, persisted as a mixture of red and green axes. Generally, only one type of pigmentation was observed in each axis, suggesting absence of individual chimeric fronds. However, green and red lateral branchlets differentiating from a single, all-red axis were detected in the 70-week-old plant (Fig. 15). Green pigmentation persisted as the branchlets enlarged (Fig. 16).

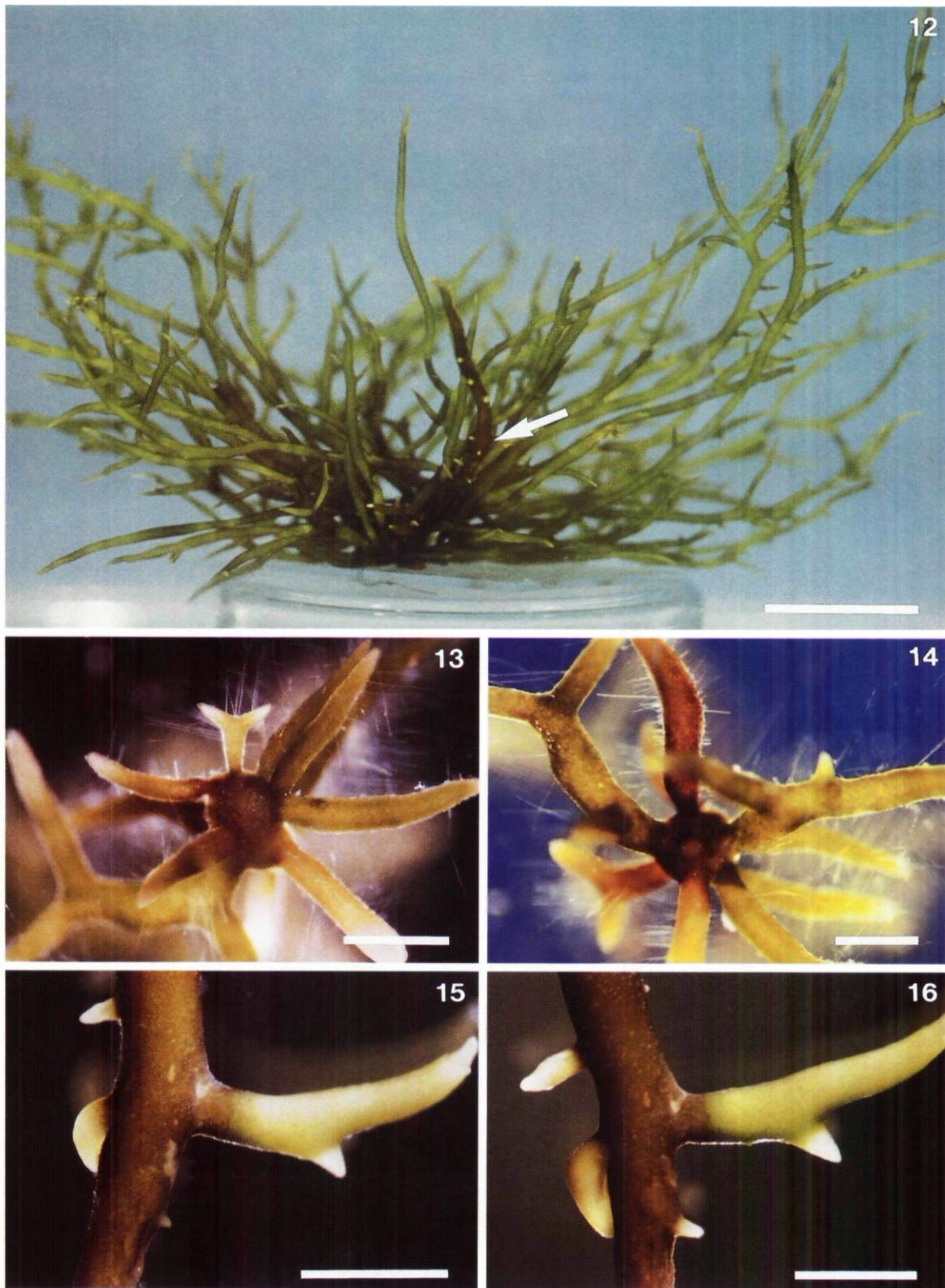
Tetraspores produced by the bicolor individual were either red or green. Germination and development of these tetraspores followed the pattern already described for the species, generating red or green plantlets.

RAPD. A total of 12 different primers were screened to compare banding patterns of one unispore (Fig. 17, upper panel) and one polyspore (Fig. 17, lower panel) thallus. All primers were positive, each producing 4–10 bands. None of the primers revealed polymorphism among the apices from the unispore thallus (Table 1), while all but one showed polymorphism among the apices from the polyspore thallus (Table 1).

Variability in growth and performance. Plotting weight increase as a function of initial weight in the three experimental plants (Fig. 18) indicated that total plant variability was greater in individuals formed by a larger number of spores. Furthermore, data dispersal and variation coefficients for an individual axis within each of the two polyspore plants (plants 13 and 3) were heterogeneous. While ramets from some axes exhibited little variation in their responses to culture conditions (e.g. axes 13.2 and 3.2, Fig. 18) others (axes 3.3 and 13.1) bore ramets that responded very differently to the culture conditions. This physiological heterogeneity was highly consistent with the chimeric nature of the individual axis observed in the bicolor thalli and the genetic polymorphism among lateral branchlets of the same erect axis detected by the RAPD technique.

DISCUSSION

Our results support the hypothesis that spore coalescence is a factor causing intraclonal variation in species with developmental patterns similar to that of *Gracilaria chilensis*. Even though adjacent spores or plantlets may coalesce during early development, intergermling fusions generally involve few cells. Thus, polyspore plantlets are formed by groups of cell clusters, each with an initial spore and its derivatives that, under TEM, appear as discrete units surrounded by a common external cell wall. Cells in adjacent clusters may fuse, establishing secondary pit plugs, but within each cluster active cell division produces numerous derivatives of the initial spore. Seemingly, none of the previous studies (Tveter-Gallagher and Mathieson 1980, Maggs and Cheney 1990) examined early developmental stages of coalescing plantlets. Rather, their contribution was to establish the continuity between coalesced, well-developed holdfasts. This is also observed in *Gracilaria*



FIGS. 12–16. External morphology and branching details of the bicolor individuals. FIG. 12. Seventy-week-old bicolor individual of *Gracilaria chilensis* grown under laboratory conditions. Most uprights are green-pigmented. Arrow indicates red-pigmented uprights. Scale bar = 2 cm. FIG. 13. Twenty-three-week-old bicolor individual of *Gracilaria chilensis* grown under laboratory conditions. Uprights with different pigmentation have grown from a bicolored holdfast. Scale bar = 2 mm. FIG. 14. The same individual as in Figure 13, 4 weeks older. The surface of the holdfast is dominated by red tissue. New red shoots are being produced. Scale bar = 2 mm. FIG. 15. Green-pigmented and red-pigmented branchlets originating from a red-pigmented upright. The upright was part of the 70-week-old bicolor individual. Scale bar = 2 mm. FIG. 16. The same branchlets in Figure 15, 1 week later. All branchlets are actively growing. Scale bar = 2 mm.

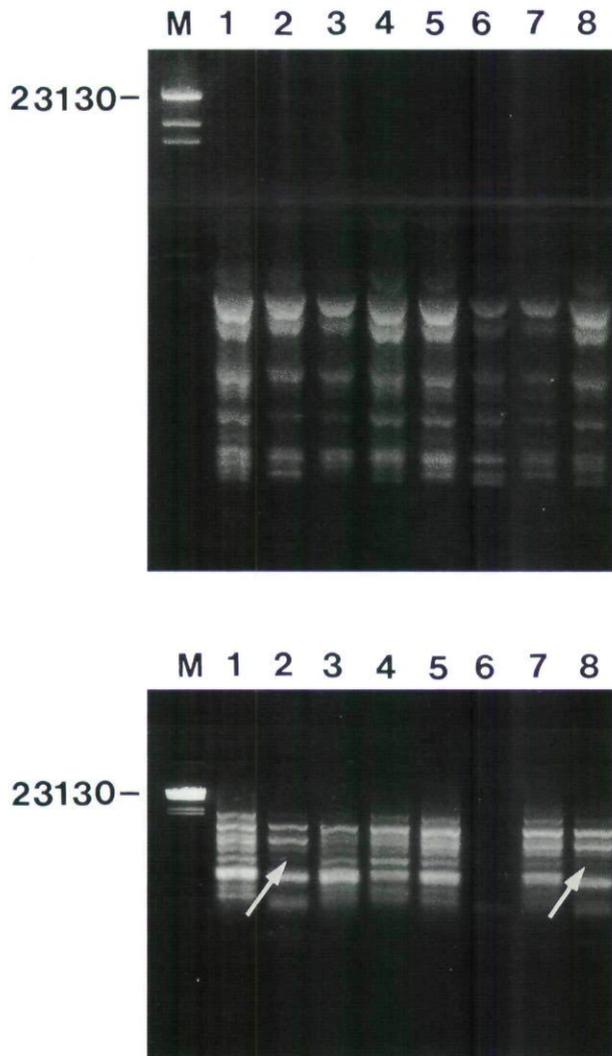


FIG. 17. RAPD patterns in monosporic and polysporic thalli. Upper panel DNA fragment patterns from a monosporic plant of *Gracilaria chilensis*. Lane numbers indicate different apices of the same thallus. Lane M corresponds to λ DNA digested with *Hind* III. Primer was OPO-06. Lower panel Polymorphic DNA fragment patterns from a polysporic plant of *Gracilaria chilensis*. Lane numbers correspond to different apices of the same thallus. Arrows indicate absence of bands. Lane M corresponds to λ DNA digested with *Hind* III. Primer was OPO-20. Sample 6 was lost during the polymerase chain reaction due to lack of mineral oil.

chilensis. However, it should be emphasized that this continuity may involve genetically different tissues united into a chimeric holdfast.

The numerical relationship between the initial spore number and the number of erect shoots is consistent with the preceding interpretation and suggests that the capacity to grow as an independent unit and eventually to form uprights is retained by the spore derivatives in the cluster. The mathematical nature of this relationship may vary from one species to another. A ratio of five spores to each shoot was suggested for large sporeling masses of *Gracilaria verrucosa* and *Chondrus crispus* (Jones 1956,

TABLE 1. Presence (+) or absence (-) of genetic polymorphism in branchlets of unisporic and polysporic thalli with various primers. Polymorphism is here defined as at least one band difference among 10 apices.

Primer	Polysporic	Unisporic
OPX-01 CTGGGCACGA	+	-
OPX-02 TTCCGCCACC	-	-
OPX-04 CCGTACCGA	+	-
OPX-06 ACGCCAGAGG	+	-
OPX-07 GAGCGAGGCT	+	-
OPX-08 CAGGGGTGGA	+	-
OPX-13 ACGGGAGCAA	+	-
OPX-18 GACTAGGTGG	+	-
OPX-20 CCCAGCTAGA	+	-
OPO-06 CCACGGGAAG	+	-
OPO-07 CAGGACTGAC	+	-
OPO-08 CCTCCAGTGT	+	-

Tveter and Mathieson 1976). A lower, nonspecific ratio was suggested for unisporic plantlets of these two species. In *G. chilensis*, we found a logarithmic relationship with proportionately fewer shoots as spore number increased. This may indicate that, as the number of spores increases, a proportionately larger number of adjacent spores fuse, not forming derivatives and, eventually, upright shoots. Alternatively, the logarithmic relationship might be an artifact in larger plantlets resulting from an increasing number of cell clusters remaining in the slide holes that were prevented from issuing uprights. Additional experiments are required to determine whether the numerical relationship is logarithmic, linear, or otherwise.

The chimeric nature of the coalesced individuals is evident in the bicolor thalli. Our results are similar to those reported by Maggs and Cheney (1990) in the sense that green and red cells in the coalesced holdfast gave origin to thalli with pigmentation similar to that of the parental cells. However, in the case of *G. chilensis*, even the individual fronds were chimeric, as evidenced by the production of green and red branchlets produced by a single all-red axis. Previous studies with bicolor individuals (Maggs and Cheney 1990) did not mention the age or the size of the studied plants; therefore, no comparisons can be made with our experiments. The detection of the chimeric frond in our cultures was done after an extended cultivation period (70 weeks). Until then, the group of green cells immersed within the red axis went undetected.

For species other than *Gracilaria*, chimeric individuals have been obtained from the fusion of genetically different protoplasts. Blades consisting of green and red groups of cells developed from the fusion products of green (mutant-type) and red (wild-type) protoplasts of *Porphyra yezoensis* Ueda (Fujita and Migita 1987, Fujita and Saito 1990). Protoplast fusion takes place only during a narrow period of time, before the potentially merging cells synthesize their cell walls. Although our TEM observations in-

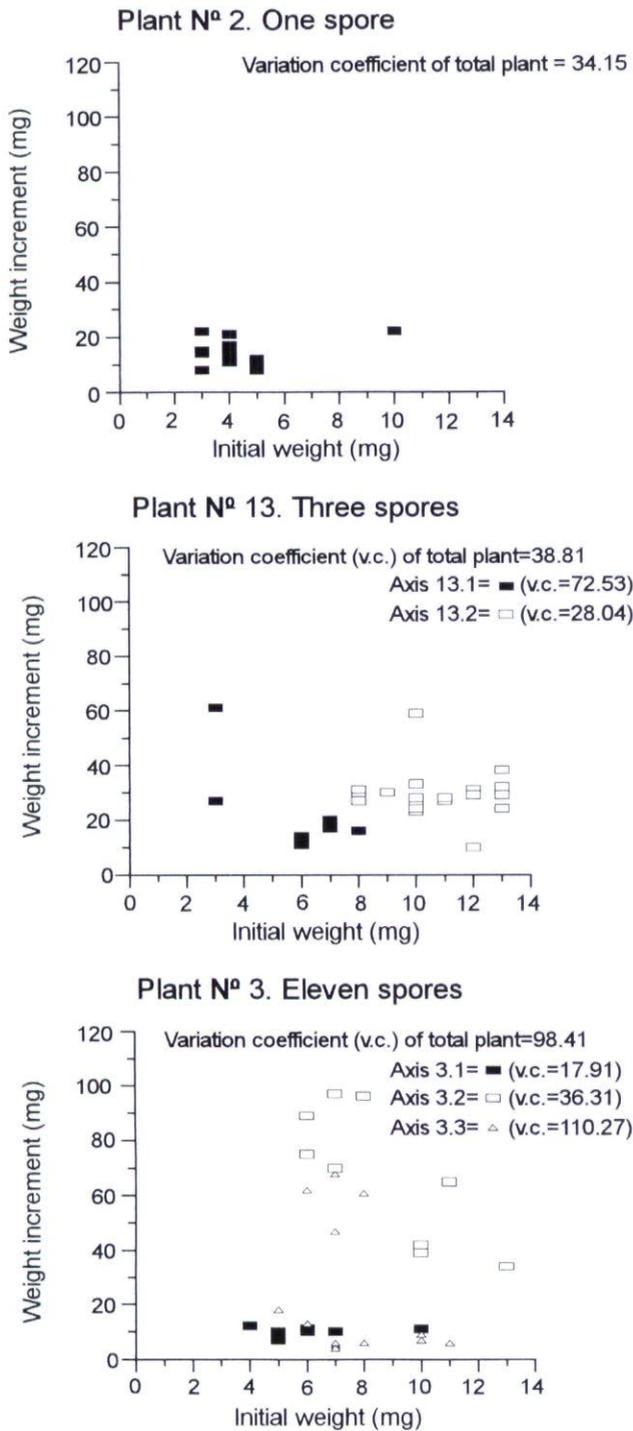


FIG. 18. Weight increase as a function of initial weight under similar abiotic conditions in ramets of thalli originated from 1, 3, and 11 coalescing spores.

indicate that spores of *G. chilensis* are naked when released, spore fusion has not been detected. Thus, it seems unlikely that our chimerical thalli resulted from spore fusion. Rather, our TEM observations of the early stages of coalescence support the notion that when the mosaic of wild- and mutant-type cells

is formed the green tissue is overgrown and hidden by the red tissue.

The monomorphic DNA patterns obtained from the unispore thallus should be considered with caution when compared to the polymorphism recorded for the branchlets from the polysporic thallus. This analysis should be replicated in a number of individuals from both origins (i.e. unispore and polysporic) in order to reach definitive conclusions about the genetic variability of both types of plants. Nevertheless, due to the consistency of our results with all the primers, the occurrence of genetically heterogeneous tissues in the branchlets of polysporic thalli is a likely phenomenon. In turn, the presence of genetically heterogeneous tissues can be interpreted as one of the factors responsible for the highly variable growth responses displayed by branchlets of the same individual when incubated under similar growth conditions. The large data dispersal and variation coefficient shown in our experiments by the polysporic thalli did not reflect high heterogeneity in all axes and branchlets. Rather, branchlets of some axes were more homogeneous while others were more heterogeneous, suggesting that genetic heterogeneity does not affect all axes in similar fashion.

Gracilaria chilensis, as many other species in the genus, is a morphologically variable taxon. Previous studies have attempted to relate morphological variability with environmental factors (for reviews, see Hoyle 1975 and Bird and McLachlan 1986), with habitat characteristics and mode of reproduction (Meneses 1996) and with genetic polymorphism. Our results suggest that the number of coalescing spores producing the thallus may be still another factor. Thalli grown from one or a few spores exhibit a single or few main axes. Thalli originated from many spores had a bushy appearance conferred by the development of many short uprights. It is as yet unknown whether or not this morphological difference persists in the field. If it does, the number of erect axes, alone or combined with the RAPD technique, could help to determine whether field-collected individuals are mono-, oligo-, or polysporic in origin.

The frequency of spore coalescence in field populations of species of *Gracilaria* and other gregarious Rhodophyta remains to be elucidated. The aggregated pattern of propagule release and dispersal, the sticky mucilaginous envelope around the spores, and the recruitment on or close to the paternal thalli with a resulting aggregated spatial distribution are all features suggesting that coalescence might be more frequent in field populations than previously thought. Because several coalescing Rhodophyta are among the dominant organisms in intertidal and shallow subtidal marine habitats (e.g. *Chondrus*, *Gigartina*, *Iridaea*, *Mazzaella*, *Sarcothalia*, *Mastocarpus*, *Ahnfeltia*, *Ahnfeltiopsis*), coalescence might not only be frequent but also ecologically important.

Several adaptive traits have been suggested for coalescing seaweeds (e.g. Maggs and Cheney 1990); however, relatively few have been quantified (e.g. Martínez and Santelices 1992, Muñoz and Santelices 1994). The most important adaptive value of clonal growth may be based on the possibility that a particular gene, or gene combination, is repeatedly exposed to a diversity of selective forces, increasing the possibility of genet survival under at least one such environment (Harper 1985). Enhanced phenotypic heterogeneity within a genet conferred by spore coalescence and chimerical tissues would result in ramets being different. This would further increase the possibilities for individual and population survival, either by increased variation and resistance to the abiotic environment (Buss 1985), by increased competitive capacity (Paine 1990), or by increased resistance to grazers (Maggs and Cheney 1990). As Buss (1985) and Paine (1990) noticed, clonal and chimeric individuals challenge the application of concepts linking Darwinian selection with population biology, and their study should help to expand traditional ecological theory.

The authors thank Drs. P. Gacesa and P. Hinrichsen and the biochemists from Instituto Nacional de Investigación Agropecuaria (INIA), Chile, for much help with the RAPD technique. Our appreciation to Dr. R. Westermeier and Mr. R. Rojas for the provision of the red- and green-pigmented fertile materials used in this study. Ms. Verónica Flores and Mr. Alejandro Muñoz helped with the ultrastructure studies and the physiological experiments, respectively. This study was supported by grant FONDECYT 1930581 to B.S. and J.C., grant SAREC-CONICYT 90-7 to B.S., and a postdoctoral grant FONDECYT 3930014 to I.M.

- Bird, C. & McLachlan, J. 1986. *Gracilaria* (Gigartinales, Rhodophyta) and productivity. *Aquatic Bot.* 26:27-49.
- Buss, L. W. 1985. The uniqueness of the individual revisited. In Jackson, J. B. C., Buss, L. W. & Cook, R. C. [Eds.] *Population Biology and Evolution of Clonal Organisms*. Yale University Press, New Haven, Connecticut, pp. 467-505.
- Dutcher, J. A. & Kapraun, D. F. 1994. Random amplified polymorphic DNA (RAPD) identification of genetic variation in three species of *Porphyra* (Bangiales, Rhodophyta). *J. Appl. Phycol.* 6:267-73.
- Fujita, Y. & Migita, S. 1987. Fusion of protoplast from thalli of two different color types in *Porphyra yezoensis* Ueda and development of fusion products. *Jap. J. Phycol. (Sōrui)* 35:201-8.
- Fujita, Y. & Saito, M. 1990. Protoplast isolation and fusion in *Porphyra* (Bangiales, Rhodophyta). *Hydrobiologia* 204/205: 161-6.
- Harper, J. L. 1985. Modules, branches and the capture of resources. In Jackson, J. B. C., Buss, L. W. & Cook, R. C. [Eds.]

- Population Biology and Evolution of Clonal Organisms*. Yale University Press, New Haven, Connecticut, pp. 1-33.
- Ho, C. L., Phang, S-M. & Pang, T. 1995. Molecular characterization of *Sargassum polycystum* and *S. siliculosum* (Phaeophyta) by polymerase chain reaction (PCR) using random amplified polymorphic DNA (RAPD) primers. *J. Appl. Phycol.* 7:33-41.
- Hoyle, M. 1975. *The Literature Pertinent to the Red Algal Genus Gracilaria in Hawaii*. Tech. Report 3, Marine Agronomy U.S. Sea Grant Program, University of Hawaii, pp. 1-340.
- Jones, W. F. 1956. Effect of spore coalescence on the early development of *Gracilaria verrucosa* (Hudson) Papenfuss. *Nature (Lond.)* 178:426-7.
- Maggs, C. A. & Cheney, D. P. 1990. Competition studies of marine macroalgae in laboratory culture. *J. Phycol.* 26:18-24.
- Martínez, E. & Santelices, B. 1992. Size hierarchy and -3/2 "Power law" relationship in a coalescent seaweed. *J. Phycol.* 28:259-64.
- McLachlan, J. 1973. Growth media-marine. In Stein, J. R. [Ed.] *Handbook of Phycological Methods. Culture Methods and Growth Measurements*. Cambridge University Press, Cambridge, pp. 25-52.
- Meneses, I. 1996. Sources of morphological variation in populations of *Gracilaria chilensis* Bird, McLachlan & Oliveira. *Rev. Chil. Hist. Nat.* (in press).
- Muñoz, A. A. & Santelices, B. 1994. Quantification of the effects of sporeling coalescence on the early development of *Gracilaria chilensis* (Rhodophyta). *J. Phycol.* 30:387-92.
- Paine, B. 1990. Benthic macroalgal competition: complications and consequences. *J. Phycol.* 26:12-7.
- Patwary, M. U., Mackay, R. M. & van der Meer, J. P. 1993. Revealing genetic markers in *Gelidium vagum* (Rhodophyta) through the random amplified polymorphic DNA (RAPD) technique. *J. Phycol.* 29:216-22.
- Patwary, M. U. & van der Meer, J. 1994. Application of RAPD markers in an examination of heterosis in *Gelidium vagum* (Rhodophyta). *J. Phycol.* 30:91-7.
- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17: 208-12.
- Santelices, B. 1992. Strain selection of clonal seaweeds. In Round, F. E. & Chapman, D. J. [Eds.] *Progress in Phycological Research*, Vol. 8. Biopress, Bristol, UK, pp. 85-116.
- Santelices, B., Aedo, D. & Varela, D. 1995. Causes and implications of intra-clonal variation in *Gracilaria chilensis*. *J. Appl. Phycol.* 7:283-90.
- Santelices, B. & Varela, D. 1993. Intra-clonal variation in the red seaweed *Gracilaria chilensis*. *Mar. Biol. (Berl.)* 116:543-52.
- Snedecor, G. W. & Cochran, W. G. 1967. *Statistical Methods*, 6th ed. Iowa State University Press, Ames, 593 pp.
- Tveter, E. & Mathieson, A. C. 1976. Sporeling coalescence in *Chondrus crispus* (Rhodophyceae). *J. Phycol.* 12:110-8.
- Tveter-Gallagher, E. & Mathieson, A. C. 1980. An electron microscopy study of sporeling coalescence in the red alga *Chondrus crispus*. *Scanning Electron Microsc.* 1980:571-80.
- Watkinson, H. R. & White, J. 1986. Some life-history consequences of modular constructions in plants. *Phil. Trans. R. Soc. Ser. B* 313:31-51.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.