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Contact responses between spores and sporelings of different species, karyological phases and cystocarps of coalescing Rhodophyta

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Abstract Coalescence is a well documented event in many red algal orders. However, it is as yet unknown if genetic compatibility and phylogenetic relationships could be factors limiting coalescence. Using controlled laboratory experiments complemented with cytological and ultrastructural analysis, in this study we test whether or not coalescence may occur between different seaweed species and between karyological phases of the same species. We also evaluate the effects of one species or karyological phase on the germination rates, germling survival and differentiation of erect axes of sporelings of a second species or phase and whether the uni- or polycystocarpic origin of the coalesced germling may affect the germination and growth or the morphology of the resulting sporeling. Results indicated that the process of coalescence is restricted to intraspecific partners only. A thick interphase with crushed cells and remains of cell walls developed in all the interspecific contacts studied. Results also indicated that coalescence may be expected between individuals of different karyological phases, as in the two cases tested *(Mazzaella laminarioides* and *Sarcothalia crispata)* the filaments of both phases grow intertwined in the new tissue of the coalesced crust. Germination rates, sporeling survival and differentiation of erect axes were all affected by the different types of experimental cultures tested. However, results suggest that allorecognition among seaweeds seems to play a minor role in coalescence. The process appears as less sensitive to genetic recognition than the cell fusion processes described for other red algal species or than the colonial fusion described for colonial invertebrates and fungi.

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Introduction

Coalescence is understood as the establishment of physical contact and growing together of two or more spores, sporelings or crustose basal portions of grown thalli in such a way that the resulting individual responds as a discrete entity and some of the original cell lines no longer retain their morphological or anatomical individuality (Santelices et al. 1999, 2003). Coalescing spores, sporelings or crusts may establish cellular connections among the neighboring cells.

Coalescence is a well documented event in many red algal orders (see Santelices et al. 1999 for review) and several benefits have been proposed for the coalesced clumps when compared with non-coalesced conspecifics. The process leads to immediate size increase in the coalesced organism. Larger size in these intertidal and shallow subtidal taxa may result in lower mortality due to herbivory or abiotic extremes and enhanced competitive performance for space (Santelices et al. 1999, 2003). Larger sizes also imply a larger photosynthetically active canopy and higher productivity. Due to the unequal growth of the erect axes and concentration of energy into a few larger blades within the coalesced clump, the onset of the first reproduction is earlier, increasing the reproductive output (Santelices et al. 1995, 1996, 1999). In addition, the coalesced clumps have greater genetic variability, which is expected to confer wider ranges of physiological plasticity.

Among animals, the fusion of different colonies to produce chimeric entities has been known since the pioneer work of Giard (1872) and Bancroft (1903). By now the process is known to occur in all groups of sessile modular animals including sponges, hydroids, corals, bryozoans and ascidians (Grosberg 1988; Hughes 1989;

Sommerfeldt and Bishop 1999). In many of these colonial animals, as in fungi, allorecognition systems govern whether somatic tissue contacts between individuals would lead to compatible fusion or to histoincompatible reactions, including rejection and non-fusion (Buss 1987, 1990; Grosberg 1988; Hidaka et al. 1997; Hart and Grosberg 1999).

In seaweeds it is as yet unknown whether or not genetic compatibility could be a factor limiting coalescence. Recent field studies have documented that the process occurs only among conspecific partners (Santelices et al. 2003), but previous findings (Maggs and Cheney 1990) have reported interspecific coalescence in laboratory cultures of species of *Gracilaria*. Furthermore, in the only study performed so far evaluating histocompatibility in grown seaweeds, González and Correa (1996) found no sign of incompatibility in experimental inter-generic grafting of fully-grown thalli of *Chondrus crispus* and *Mazzaella laminarioides*. Using controlled laboratory cultures complemented with morphological, cytological and ultrastructural analysis, in this study we test whether or not coalescence may occur between different species or between different karyological phases of the same species (e.g. tetrasporophytes and gametophytes). We also evaluate the effects of one species or karyological phase on the germination rates of spores, germling survival and differentiation of erect axes of sporelings of a second species or phase. Also we tested whether or not the uni- or polycystocarpic origin of the spores may affect the germination and growth of the resulting sporeling.

Materials and methods

General experimental procedures

Carpospores and carposporelings of *Gracilaria chilensis* Bird, McLachlan and Oliveira, *Mazzaella laminarioides* (Bory) Fredericq, *Nothogenia fastigiata* (Bory) Parkinson and *Sarcothalia crispata* (Bory) Leister were used to test for interspecific effects on coalescence, germination and growth. Carpospores and tetraspores of *M. laminarioides* and *S. crispata* were used for interphase testing, while carpospores of *M. laminarioides* were used to compare unicystocarpic with polycystocarpic sporelings. Fertile thalli of these species were collected between April 1999 and February 2002 in Maullin, Southern Chile (41°36′S, 73°36′W) and Matanza, Maitencillo and Topocalma, all localities in Central Chile (32–34°S). Collected thalli were maintained at 4°C in coolers for 3–6 h while they were being transported to the laboratory. In the laboratory, thalli were washed with running tap water and maintained for 24 h in filtered seawater under constant temperature (14 ± 2°C), irradiance (20 ± 10 μmol photons·m^{-2} s^{-1}) and photoperiod (12:12 LD). Sporulation was induced by immersing mature cystocarps in microfiltered seawater (0.45 μm) after 24 h of dehydration of the fertile thalli at room temperature (15 ± 2°C).

The methodology after spore shedding differed according to the experiment and specific details are given in the following sections.

Cytological and ultrastructural studies

Characterization of the process of encounter between the different spores and/or sporeling at the cellular level followed the methods described in previous studies (Santelices et al. 1996, 1999). Spores released by any given phase or species were allowed to settle on one side of a plastic cover slip (EMS 72260) while the spores of a second species or phase were deposited 30–50 μm away. All slides were individually incubated in Petri dishes (50×10 mm), filled with SWM-3 culture medium (McLachlan 1973) and maintained under the laboratory conditions described above. The culture medium was changed weekly. Sporeling growth was monitored every 3 days until spores upon a plate eventually made contact. All tissue and culture manipulations were done in a laminar flow hood and glass materials were autoclaved prior to their use. The various stages of sporeling development included in this study are the result of incubations lasting up to 500 days.

Freshly released spores and the subsequent stages of development were fixed for ultrastructural analysis following the protocol described elsewhere (Santelices 1996, 1999). Spore fixation in the glutaraldehyde-acrolein mixture in SWM-3 culture medium was followed by postfixation in an osmium-potassium ferricyanide mixture. Sporelings were fixed at room temperature in 5% glutaraldehyde and 3% acrolein in SWM-3 culture medium for 3 h under vacuum. A longer fixation protocol was used for older sporelings, consisting in 3 days at 5°C, with daily changes of the fixative solution. Dehydration, embedding, and infiltration were standard (see Santelices et al. 1996, 1999). Thin sections were stained with 4% uranyl acetate and lead citrate according to Reynolds (1963) and were observed with a JEOL 100SX electron microscope operated at 60 kV.

Interspecific effects on germination and growth

In these experiments, the germination rates, rates of sporeling survival and differentiation of erect axes of sporelings in bispecific cultures were compared with their respective unispecific controls. The bispecific cultures were *M. laminarioides* with *G. chilensis, M. laminarioides* with *N. fastigiata* and *M. laminarioides* with *S. crispata*. The three species frequently are found living together in middle and low intertidal habitats of central and southern Chile (Hannach and Santelices 1985; Santelices 1990; Santelices et al. 2003).

Carpospores released by several conspecific mature cystocarps were mixed and deposited in holes (1.8 mm diameter, 250–300 μm deep) drilled in glass microscope slides. Since previous studies (Santelices et al. 1999) have shown that germination and germling growth may vary according to the number of spores conforming the sporeling, the number of spores placed in each hole ranged, in multiples of 2, from 4 to 20. To compare germination rates and germling survival between unispecific and bispecific cultures, half the number of spores used in the corresponding unispecific cultures were replaced by an equal number of spores of a second species. Since differentiation of erect axes is a function of the original number of conspecific spores in the germling (Santelices et al. 1999), number of axes in bispecific cultures were compared with unispecific controls having half the number of spores only. For example, a 20-spore bispecific culture of *M. laminarioides-G. chilensis* contained 10 spores of each species. These were compared with a 10-spore conspecific culture. Interspecific comparisons on spore germination and sporeling survival included 27 treatments. The factor ''number of spores'' had nine levels (e.g. 4, 6, 8, 10, 12, 14, 16, 18 and 20 spores). Results of treatments with 2 spores were very variable and therefore excluded from further analysis. The factor ''number of species'' included three levels (the bispecific cultures and the respective unispecific culture of each species). Six replicate slides were used in each one of the 27 treatments.

In the case of interspecific comparisons of erect axes differentiation, the number of levels of the factor ''number of spores'' was reduced to 4 (e.g. 8, 12, 16 and 20 spores in bispecific cultures and 4, 6, 8 and 10 spores in unispecific cultures). Thus, in these experiments the total number of treatments was 12, each with 6 replicates.

All slides were individually incubated in Petri dishes (50×10 mm), filled with SWM-3 culture medium (McLachlan 1973) and maintained under controlled culture conditions of temperature

($14 \pm 2°C$), irradiance (45 ± 10 μmol photons$\cdot m^{-2} \cdot s^{-1}$) and photoperiod (12:12 LD). Culture medium was changed weekly. Germination rate (percent) was measured after 96 h of incubation, sporeling survival after 15 days and number of erect axis emerging from each germling counted after 30 days of incubation.

Data on germination and survival rates were arcsine transformed. Then, these and the data on number of erect axes were tested for homogeneity of variance using Cochran's test and for normality using Shapiro-Wilks' W test. Since data satisfied both requirements, results were then compared using a Model I of two way ANOVA where the two factors are (1) uni- and bispecific results and (2) number of spores sown. Whenever significant differences among main factors were found, the factorial ANOVA was followed by a posteriori (Tukey) tests (Snedecor and Cochran 1967; Sokal and Rohlf 1969).

Interphase effects on germination and growth

Tetrasporic and cystocarpic thalli of *M. laminarioides* and *S. crispata* were used to test whether coalescence may occur among different karyological phases of a given species. The experimental set up and the statistical procedures used were similar to the above experiments, using different phases rather than different species. However, number of erect axes was not counted because the embryonic origin (tetrasporic versus cystocarpic) of the erect axes often could not be properly traced in the biphasic germlings.

Uni- and polycystocarpic sporelings

Only carpospores of *M. laminarioides* were used in these experiments. Carpospores from six different cystocarps in six different genets were mixed together in a seawater suspension and used in the polycystocarpic treatments. All materials for the unicystocarpic treatments and replicates were collected from a single, mature cystocarp. All other experimental procedures were as described above for interspecific comparisons.

Results

Cytological and ultrastructural studies

Sporelings of the same or different species made contact with each other during early development (Fig. 1A, B). Contact between conspecific sporelings established morphological continuity among them (e.g. long arrow in Fig. 1A). In contrast, interspecific partners maintained morphological individuality which, at low resolution (short arrows in Fig. 1A, B), appears as a clear line between the two contacting discs. In stained sections (Fig. 1C), the contacting fronts of these interspecific encounters consist of compressed cells and cell remains with the outer cell walls deeply stained.

During later stages of development, the four experimental species exhibited radial expansion of the basal crust. The crustose tetrasporophyte of *Nothogenia fastigiata* did not differentiate erect axes (N.f. in Fig. 1D) while those of *Mazzaella laminarioides* (M.l. in Fig. 1D, E), *Sarcothalia crispata* (S.c. in Fig. 1E) and *Gracilaria chilensis* (G.ch.) differentiated erect axes as the crust expanded. *S. crispata,* however, showed a proportionately larger development of the erect axes from rather narrow bases. Thus, whenever two interspecific crusts of *N. fastigiata, M. laminarioides* or *G. chilensis* met each other, they formed contacting fronts (e.g. arrows in Fig. 1D). In the case of encounters involving *S. crispata* (Fig. 1E), its basal crusts often appeared surrounded by the growing borders of the crusts of any of the other three species (e.g. arrows in Fig. 1E).

Under low magnification, sections through the contacting fronts of interspecific crusts always showed the presence of a thick interface formed by compressed, necrotic cells and by cell remains of one or both interacting species (Fig. 1F). Under TEM, the cells closer to the contacting zones may appear compressed and with abnormal cell structure (Fig. 2A), while cells farther beyond the contacting front exhibited a normal morphology (Fig. 2B). In addition, secondary pit connections between cells of different species were absent, even when they were in close proximity (Fig. 2C).

When the crusts of different karyological phases of *M. laminarioides* or *S. crispata* contacted each other, they grew together without the formation of a necrotic interphase. In about 20% of the coalescing sporelings a healing scar was seen in the contacting zone (arrow in Fig. 1G). However, after a few (3–6) months of growing together, the anatomical continuity of the crust was established. After this time, new erect axes may appear in what used to be the contact zone between the two crusts.

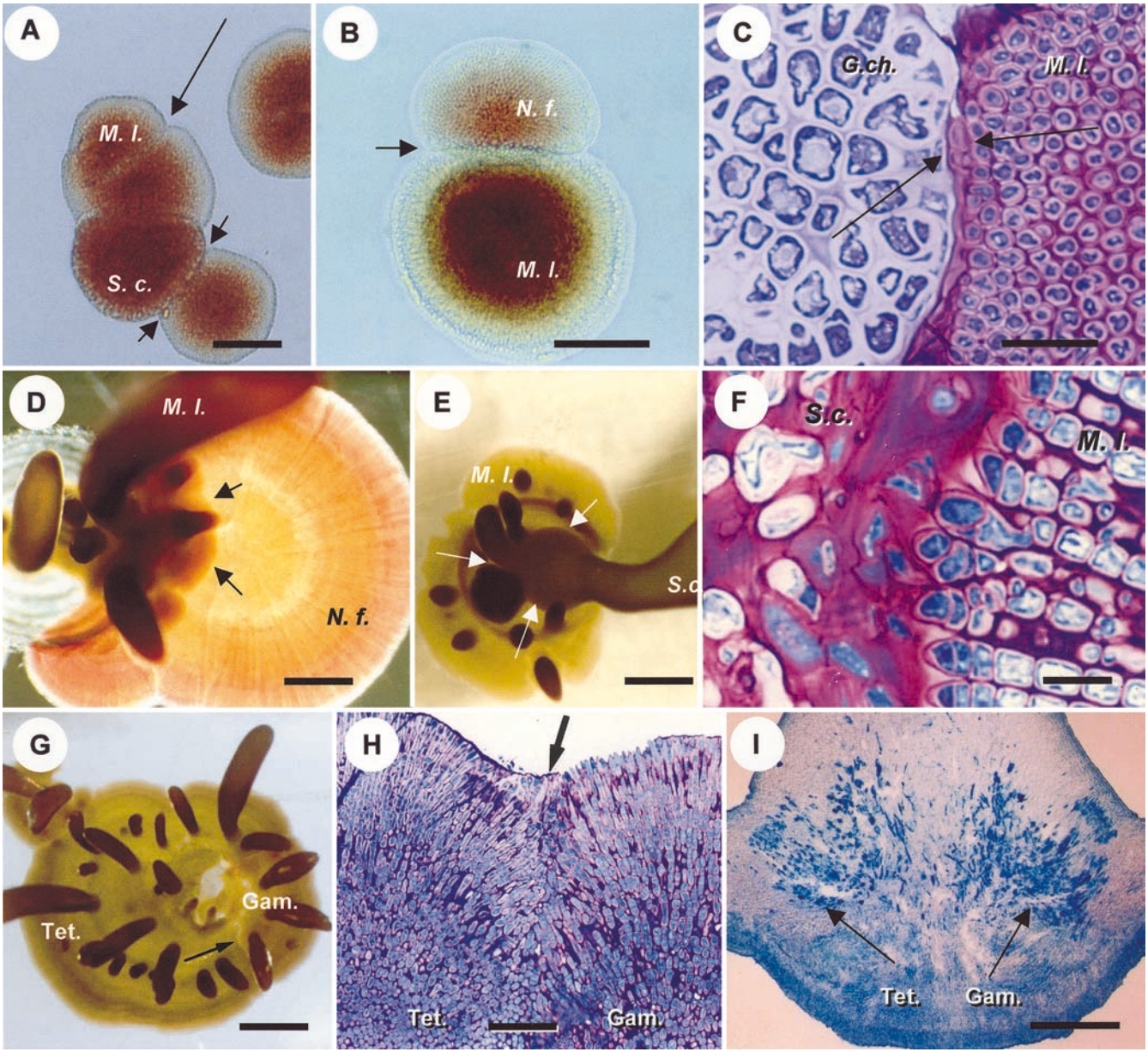
Examination of the contacting tissues of the two karyological phases showed, in both species, an absence of a thick interphase with necrotic tissues (Fig. 1H, I), although cell wall remains of crushed cells were seen in the healing areas. Sections through the new growth in the coalesced holdfasts of *M. laminarioides* (Fig. 1H) showed intertwining of growing filaments in the contact zone (arrow in Fig. 1H). In the case of *S. crispata*, the original sporelings appeared as growth centers, projecting filaments in all directions (arrows in Fig. 1I). In neither case, no distinction between the two karyologically different crusts could be found in the more peripheral tissues produced after coalescence.

Interspecific effects

M. laminarioides and *G. chilensis*

A comparison of the percent germination of spores between uni- and bispecific cultures of *M. laminarioides* and *G. chilensis* indicates that the presence of one species may negatively affect germination of the other species (Fig. 3A, B). Differences between uni- and bispecific cultures of *M. laminarioides* are statistically significant (ANOVA, $F = 6.273$; $P = 0.0141$), but differences between number of spores sown were not statistically significant.

Although germination rates in unispecific cultures of *G. chilensis* were higher than in bispecific cultures, these differences were not significant (ANOVA, $F = 0.544$;



$P = 0.463$), neither were the differences between number of spores sown ($F = 1.972$; $P = 0.068$).

The presence of sporelings of *G. chilensis* also significantly reduced the survival rates of sporelings of *M. laminarioides* (Fig. 3C). Differences between unispecific and bispecific cultures (ANOVA, $F = 23.197$; $P < 0.001$), between number of spores sown ($F = 3.613$; $P = 0.132$) and the interaction between the two main factors ($F = 2.188$; $P = 0.0355$) are all significant. Application of Tukey test indicate significant differences among unispecific and bispecific cultures in one of the individual treatments (e.g. eight spores sown; Tukey test, $P < 0.0185$). By contrast, germling survival of sporelings of *G. chilensis* in uni- and bispecific cultures with *M. laminarioides* are essentially similar (Fig. 3D), suggesting lack of effects of sporelings of *M. laminarioides* on the survival of *G. chilensis*.

In both species, the average number of erect axes developed from the sporelings is significantly affected by the presence of sporelings of the other species (Fig. 3E, F; ANOVA, $F = 11.464$; $P < 0.003$ for *M. laminarioides;* $F = 23.198$, $P < 0.001$ for *G. chilensis)*. Although in each species the number of erect axes issued in unispecific cultures increases with the increasing number of spores sown (Fig. 3E, F), differences between number of spores sown is non-significant ($F = 0.676$; $P = 0.574$). The trend of increasing the number of erect axes as a function of the number of spores sown is not shown by the sporelings of either species in bispecific cultures.

M. laminarioides and *N. fastigiata*

The presence of spores and sporelings of *N. fastigiata* significantly increases the germination percent (Fig. 4A,

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Fig. 1A–I Morphological and ultrastructural observations on contact reactions between spores and sporelings of different species and karyological phases under controlled laboratory conditions. **A** Intraspecific coalescence (*long arrows*) and interspecific contacts (*short arrows*) in *Mazzaella laminarioides* (*M.l.*) and *Sarcothalia crispata* (*S.c.*) in 10-day-old sporelings. *Scale bar* 50 μm. **B** Interspecific contact (*arrow*) between 30-day-old sporelings of *Nothogenia fastigiata* (*N.f.*) and *M. laminarioides* (*M.l.*). *Scale bar* 100 μm. **C** Longitudinal section through 30-day-old contacting sporelings of *G. chilensis* (*G.ch.*) and *M. laminarioides* (*M.l.*) stained with toluidine blue. Note the thick interface between both sporelings (*arrows*). *Scale bar* 20 μm. **D** Contacting fronts (*arrows*) of 5-month-old, radially expanding crusts of *M. laminarioides* (*M.l.*) and *N. fastigiata* (*N.f.*). Note the pink crusts of *N. fastigiata* and uprights of different lengths in *M. laminarioides*. *Scale bar* 500 μm. **E** Narrow-based, 3-month-old sporeling of *S. crispata* (*S.c.*) surrounded by the radially expanding, 3-month-old crust of *M. laminarioides*. Note the uprising of different lengths produced by both species and the thin interspecific contact area (*arrows*). *Scale bar* 2 mm. **F** Longitudinal section through the contacting zone between 3-month-old crusts of *S. crispata* (*S.c.*) and *M. laminarioides* (*M.l.*). Note the thick interface with cell remains. Sections are stained with toluidine blue. *Scale bar* 10 μm. **G** Six-month-old germling formed by coalescence of a gametophyte (*Gam.*) and a tetrasporophyte (*Tet.*) of *M. laminarioides*. Coalescence occurred when the individual discs were 1 month old. Note the healing scar (*arrow*). *Scale bar* 1 mm. **H** Longitudinal section through a 6-month-old biphasic germling of *M. laminarioides* stained with toluidine blue. Note the lobes formed by the radial growth of the external meristematic filaments of each phase and the slight depression formed in the contact zone (*arrow*) with intertwining of filaments. *Scale bar* 100 μm. **I** Perpendicular section through a 500-day-old biphasic germling of *S. crispata* stained with aniline blue. Note that the coalesced sporeling has two radiating growth centers formed by the two original germlings (*arrows*). No evidence of necrotic reaction is seen in the contact zone. *Scale bar* 200 μm

ANOVA, $F = 30.252$, $P < 0.001$) of *M. laminarioides,* but differences among number of spores swon are non-significant (ANOVA, $F = 1.346$; $P = 0.2312$). The presence of sporelings of *N. fastigiata* does not significantly affect sporeling survival of *M. laminarioides* (Fig. 4C; ANOVA, $F = 1.014$; $P = 0.316$) but it significantly increases differentiation of their erect axes (Fig. 4D; ANOVA, $F = 8.4125$, $P < 0.007$). In this last experiment, differences between the number of spores sown (ANOVA, $F = 6.728$, $P < 0.0009$) and the interaction between the two main factors ($F = 7.4737$; $P < 0.0005$) were significant. Application of a posteriori (Tukey test) indicates significant differences ($P = 0.0007936$) in number of erect axes between unispecific and bispecific treatments started with 16 spores.

The presence of spores of *M. laminarioides* did not significantly affect the germination rates of *N. fastigiata* (Fig. 4B, ANOVA, $F = 2.674$; $P = 0.106$), however, it decreased the germination survival of the latter species (Fig. 4D). Differences between unispecific and bispecific cultures (ANOVA, $F = 26.734$, $P < 0.0001$), number of spores sown (ANOVA, $F = 2.240$; $P = 0.032$) and the interaction between these two factors (ANOVA, $F = 3.294$; $P = 0.002$) were all significant. A posteriori (Tukey) tests indicate significant differences in the number of erect axes differentiated between unispecific and bispecific cultures in two individual comparisons

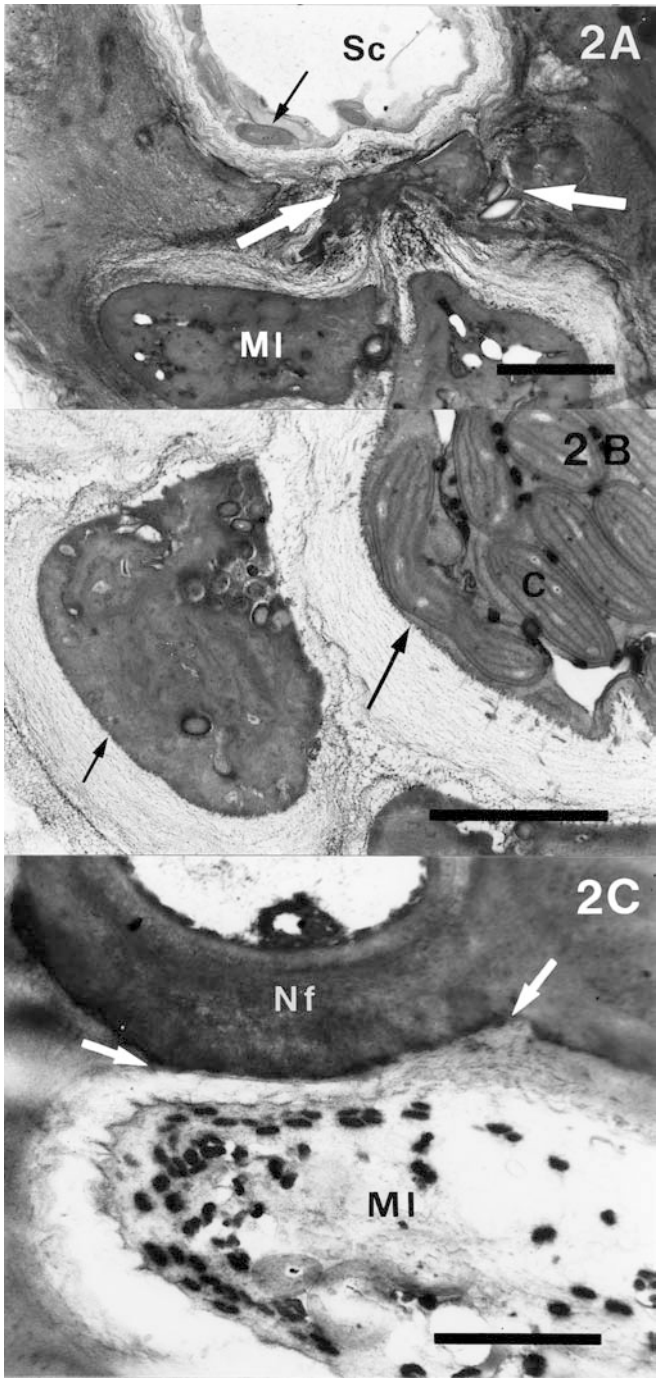

Fig. 2A–C Ultrastructure of carpospores of contacting partners. **A** Cell and cell wall remains of 5-month-old sporelings of *M. laminarioides* (*M.l.*, *white arrows*) in close contact with cells of *S. crispata* (*S.c.*). Note the normal shape of chloroplasts in the contacting cells of this last species (*black arrow*). *Scale bar* 3 μm. **B** Abnormal (*short arrow*) and normal (*long arrow*) cell structure in 20-day-old germlings of *G. chilensis* in close contact with germlings of *M. laminarioides*. *Scale bar* 2 μm; *c* chloroplast. **C** Close contact between cells of *N. fastigiata* (*N.f.*) and *M. laminarioides* (*M.l.*) in 3-month-old germlings. Note the clear limits between both cell walls (*arrows*) and the lack of secondary pit connections. *Scale bar* 2 μm

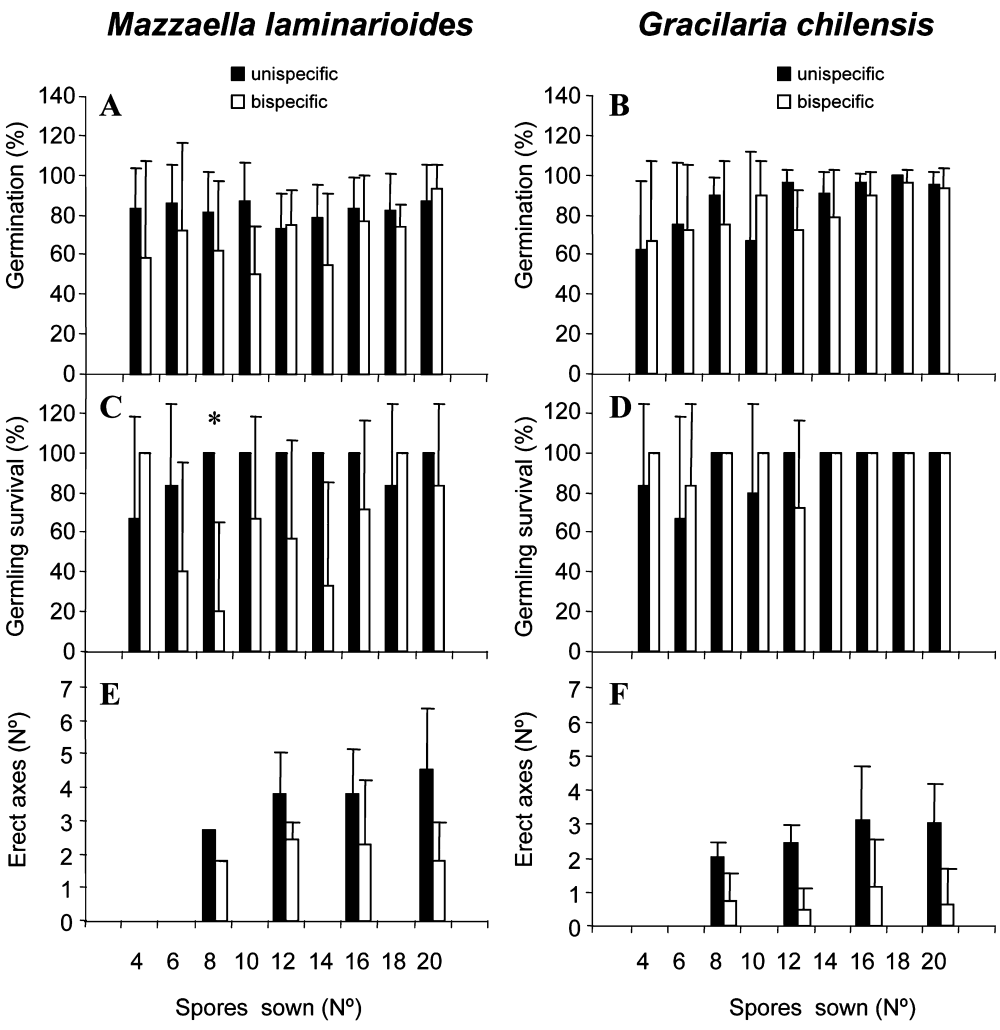

Fig. 3A–F Germination rates, sporeling survival and differentiation of erect axes in uni- and bispecific cultures of *M. laminarioides* and *G. chilensis*. *Bars* are SE; the *asterisk* above some of the comparisons indicates significant differences between treatments

(Fig. 4E, $P < 0.022$ for 14 spores and $P = 0.042$ for 16 spores).

M. laminarioides and *S. crispata*

Germination rates of *M. laminarioides* were higher (Fig. 5A) in unispecific cultures than in bispecific cultures with spores of *S. crispata* (ANOVA, $F = 21.837$, $P < 0.0001$), but differences among spores sown are non-significant (ANOVA, $F = 0.0124$; $P = 0.5100$). The spores of *S. crispata* also germinate less in bispecific than in unispecific cultures (Fig. 5B). However, differences are not significant due to the variability in germination rates exhibited by this species.

Germling survival (Fig. 5C, D) and number of erect axes issued (Fig. 5E, F) both in *M. laminarioides* and *S. crispata* are affected by the presence of a second species. In almost all the treatments, survival and axis differentiation was higher in unispecific than in bispecific cultures. Furthermore in some cases (e.g. germling survival of *S. crispata* in the treatment with eight spores, Fig. 3D), differences between unispecific and bispecific treatments were very large. However, in all cases differences were not statistically significant due to data dispersal.

Interphase effects

M. laminarioides

The presence of tetraspores of *M. laminarioides* decreased the germination rate of carpospores of the same species in seven out of the nine treatments tested (Fig. 6A) and differences between uni- and bispecific cultures are significant (ANOVA, $F = 5.474$; $P < 0.022$). A reciprocal effect with the germinating tetraspores was not found (Fig. 6B). In the latter case, some of the individual treatments exhibited higher germination rates than the respective control, but they correspond irregularly to uniphasic or to biphasic cultures.

Mixed cultures of carpospores and tetraspores did not significantly affect germling survival in any of the two phases under cultivation (Fig. 6C, D; ANOVA $F = 2.697$; $P = 0.104$, for carpospores; $F = 1.832$; $P = 0.129$, for tetraspores). In all these experiments, the differences

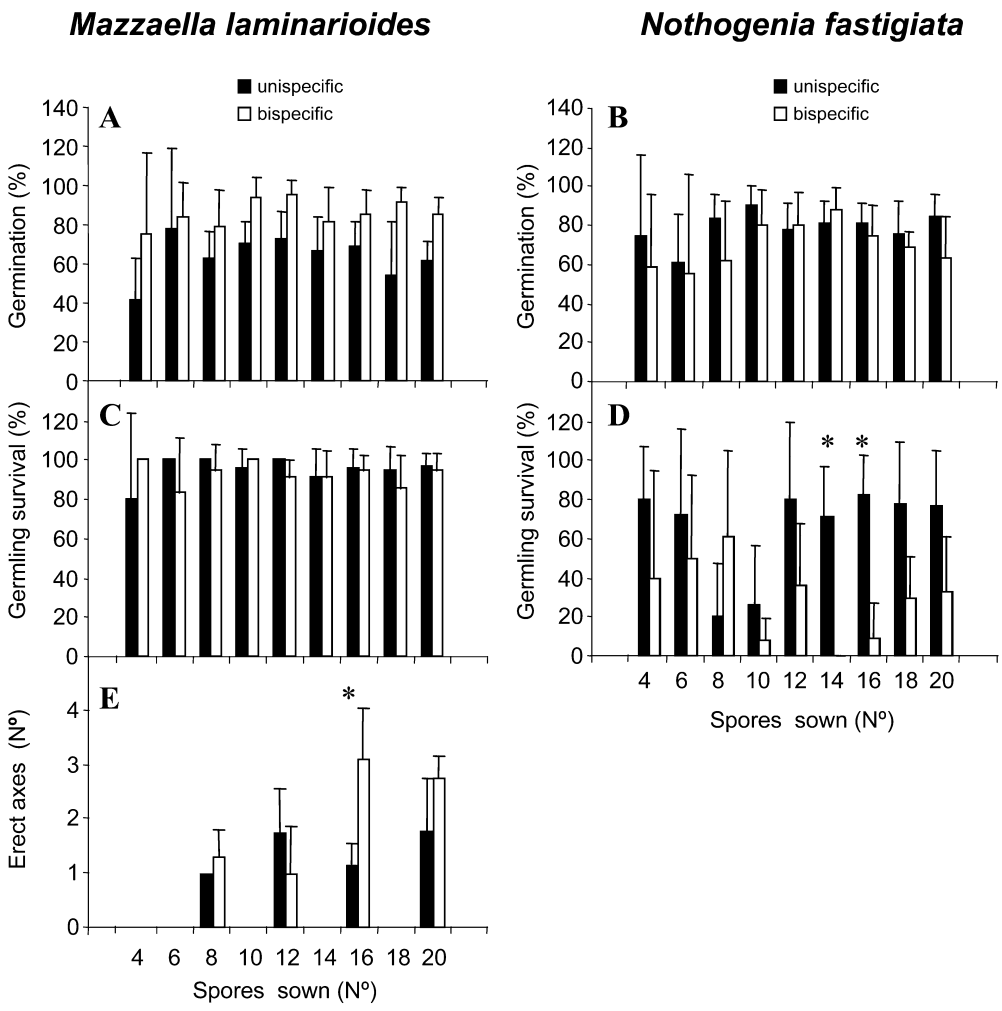

Fig. 4A–E Germination rates, sporeling survival and differentiation of erect axes in uni- and bispecific cultures of *M. laminarioides* and *N. fastigiata*. *Bars* are SE; the *asterisk* above some of the comparisons indicates significant differences between treatments

between the number of spores sown or the interactions between the two main factors were non-significant.

S. crispata

The germination rates of carpospores (Fig. 7A) and tetraspores (Fig. 7B) of *S. crispata* were low and variable, both in uniphasic and in biphasic cultures. However, carpospore germination rates are significantly higher (ANOVA, $F = 5.248$, $P = 0.025$) in uniphasic than in biphasic cultures, while the opposite is true (ANOVA, $F = 9.431$, $P < 0.003$) for tetraspores. A similar pattern is found when germling survival rates are compared (Fig. 7C, D). In this last case, however, only the differences with tetrasporic cultures are statistically significant (Fig. 7D; ANOVA, $F = 8.348$; $P < 0.046$). Differences between number of spores sown or the interactions between the two main factors were non-significant in all these experiments.

Uni- and polycystocarpic cultures

Germination rates of cultures with unicystocarpic spores of *M. laminarioides* were significantly lower than polycystocarpic cultures (Fig. 8A; ANOVA, $F = 8.194$; $P = 0.005$). These experiments showed no differences in germling survival between uni- and polycystocarpic treatments (Fig. 8B) while differentiation of erect axes was significantly higher (ANOVA, $F = 4.311$; $P = 0.04$) in unicystocarpic than in polycystocarpic coalescing germling. Differences between number of spores sown or the interactions between the two main factors were non-significant.

Discussion

The presence of an interphase with crushed cells and cell wall remains in all the interspecific contacts examined suggests that the process of coalescence is restricted to intraspecific partners only. A similar result was found by Santelices et al. (2003) in recent field studies of coalescence.

Our results also indicate that coalescence could be expected between individuals of different karyological phases within a given species. Early stages of these fusions may exhibit a few crushed cells and cell remains within the two approaching crusts. Later stages, however, indicate that the filaments of both phases may grow intertwined in the new tissue of the coalesced crust,

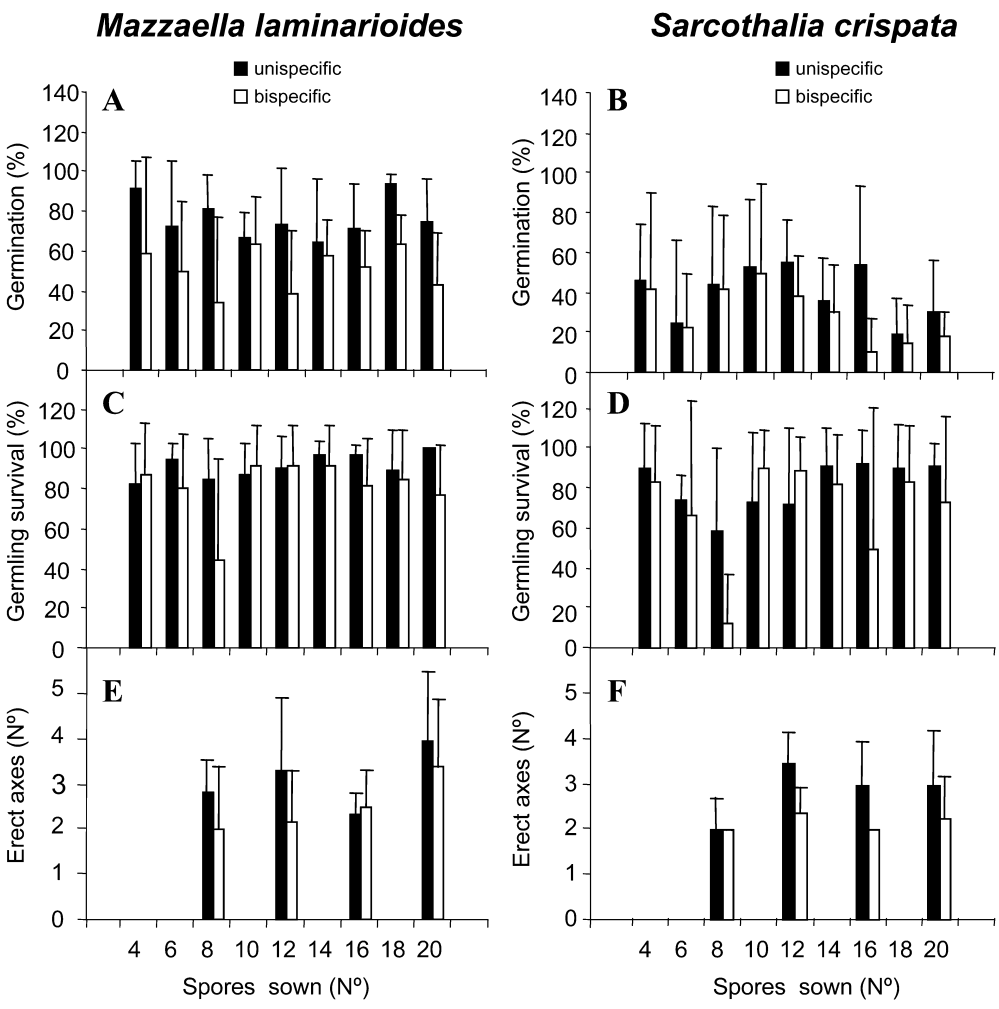

Fig. 5A–F Germination rates, sporeling survival and differentiation of erect axes in uni- and bispecific cultures of *M. laminarioides* and *S. crispata*. *Bars* are SE

without any evidence of separation between the two phases.

Although the present results describe the fate of interspecific and interphase contacts between crusts, they do not explain the mechanisms avoiding coalescence among species. In our cultures the thick interphase with necrotic tissues and cell wall remains started forming a few weeks after sporelings made contact with each other. The interphase was already well established in young sporelings (3–6 months old) and it was very noticeable among older contacting holdfasts (12–15 months old). A morphologically similar necrotic zone was found among contacting holdfasts of *Mazzaella laminarioides* and *Nothogenia fastigiata* in the field (Santelices et al. 2003) and it seems to correspond to a general response among interspecific partners before one overgrows the other.

Interspecific differences in growth rates could explain how the tissue of one partner could compress and eventually crush the contacting cells of the other partner. However, other factors such as histocompatibility also could be involved. The responses between contacting interspecific partners in our experiments are similar to incompatibility responses exhibited by some land plants after grafting. In the case of *Sedum telephoides* and *Solanum pennelli,* the contacting cells first showed losses in organelle integrity. Then, there is cellular death followed by the formation of a necrotic interphase with abundant cell remains (Moore and Walker 1983). Seemingly there are no experimental data supporting interspecific histocompatibility responses among the seaweeds. However, different geographic isolates of *Griffithsia pacifica* exhibited incompatibility during cell fusion, causing cytoplasmic swelling, protoplasm agglutination and chloroplast fusion and even degradation in the more sensitive strains (Koslowsky and Waaland 1984, 1987).

An additional explanation for the lack of coalescence among neighboring individuals of different species could be advanced from recent information on algal defensive responses and recognition. It is now known that oligosaccharides are involved in signaling and recognition in the red algae *Chondrus crispus* (Bouarab et al. 1999, 2001; Potin et al. 1999) and *Gracilaria conferta* (Weinberger et al. 1999, 2001; Weinberger and Friedlander 2000). The oligosaccharides are the result of cell wall degradation of the interacting species (Potin et al. 2002), a response likely to occur when two crusts make contact

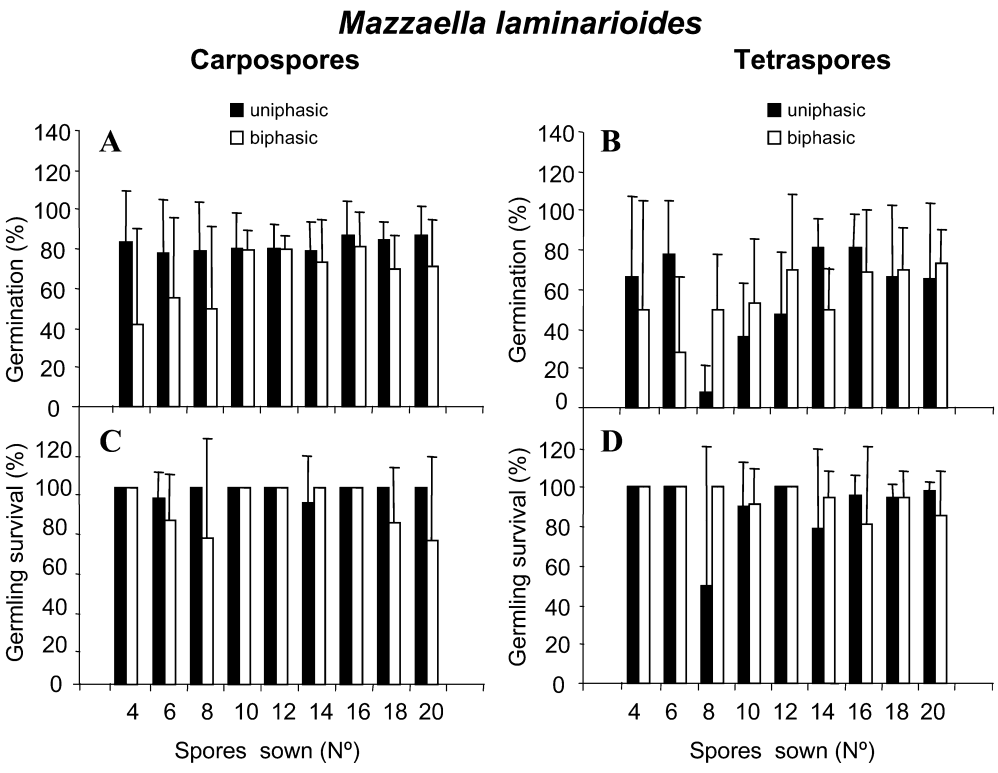

Fig. 6A–D Germination rates and sporeling survival in uni- and biphasic cultures of *M. laminarioides*. *Bars* are SE

and trigger the local destruction of cells by release of cell-wall degrading enzymes. Thus, it is certainly possible that oligosaccharides are released during the destruction of cells at the beginning of the contacts and serve as signals to be recognized or not by the interacting individuals. As a result of the biochemical communication, identifying fragments of oligosaccharides as "self" would render a compatible reaction and a successful coalescence. By contrast, fragments might not be recognized as "self" and the result could vary from a failure to establish a coalescing unit to trigger uni- or bidirectional defense responses that, instead, could cause disfunction or even death of one of the partners.

The thick, necrotic interface developed between the contacting cells of different species often implies a distance too large and often filled with cell wall remains to be crossed by secondary pit connections. However, in the early stages of the development of this interface, intact cells of different species could be seen in close proximity (e.g. Fig. 2D). Yet, no pit connections were observed in these cases, suggesting that other processes, such as cell-to-cell recognition, might be involved in the establishment of these connections.

On the other hand, none of the above reactions were observed in mixed cultures involving two phases of the same species. Since the two phases of each of the species tested differed in cell wall composition (K carrageenan in gametophytes and λ carrageenan in tetrasporophytes; McCandless et al. 1973; Matsuhiro and Zanlungo 1976a, 1976b; Craigie 1990) incompatibility responses and necrotic tissues were also expected in the contact fronts between both phases, but they did not occur. Some cell wall remains could be found in the healing scars resulting from early fusion between phases. However, no evidence of cell mortality was found in the later development of the coalesced holdfasts. Pit connections were observed in the new filaments growing in the contact zone. However additional studies using special techniques for nuclear genome characterization (e.g. Kapraun et al. 1994) are needed to unequivocally conclude whether secondary pit connections can be established among haploid and diploid cells.

The possibility of coalescence among karyologically different phases may constitute a potential explanation for the observed occurrence of gametophytes and sporophytes arising from a single holdfast in the field. Kain and Destombe (1995) anticipated this possibility in populations of the genus *Gracilaria*. More recently, working with an intertidal population of *M. laminarioides* from Central Chile, Faugeron (2002) found 2–3% of the almost 400 clumps studied to be formed by blades of both phases arising from a single holdfast.

In addition to coalescence, germination rates, sporeling survival and differentiation of erect axes were affected by the different types of experimental cultures tested, as compared to their respective controls. Germination rates could be reduced (e.g. germination of *M. laminarioides* when co-cultured with *G. chilensis)* or increased (e.g. spores of *M. laminarioides* co-cultured with *N. fastigiata)* compared to their respective unispecific cultures. However, the precise mechanisms inducing these differences in these species are unknown. Interactions between species and phases may derive from ectocrines and allelopathic substances released by

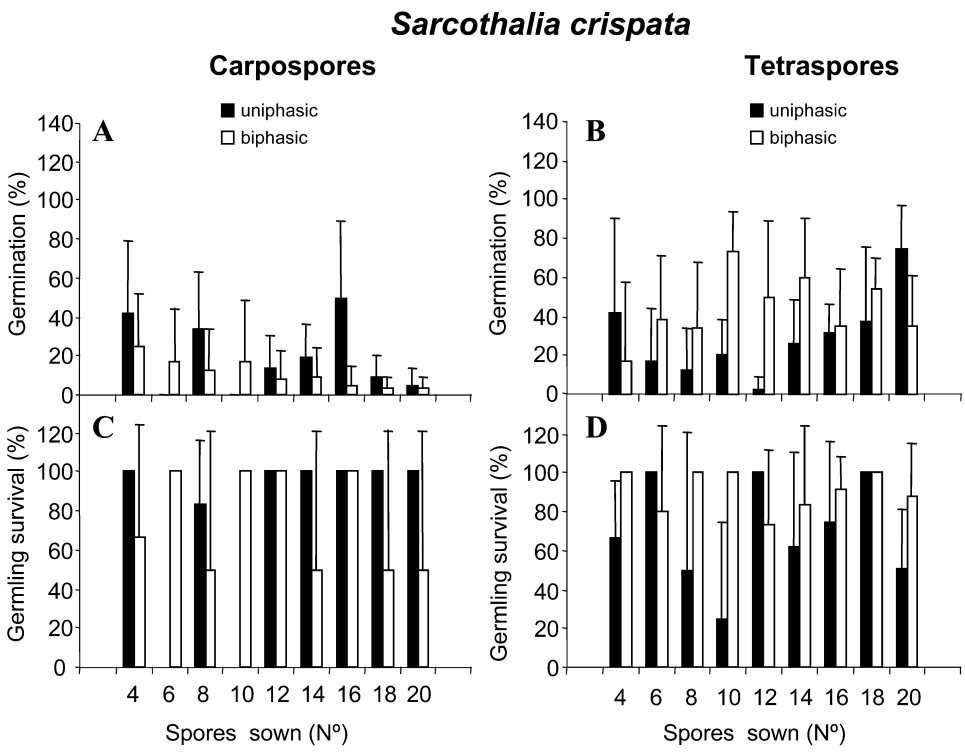

Fig. 7A–D Germination rates and sporeling survival in uni- and biphasic cultures of *S. crispata*. *Bars* are SE

germinating propagules that may either negatively or positively affect the germination rates of other species. Such negative interactions have been documented among brown and red algal species (Conover and Sieburth 1996; Huang and Boney 1983, 1984) while positive interactions among conspecific propagules of *Enteromorpha* (Callow et al. 1997) and conspecific propagules of red algae (West, in Callow et al. 1997) have been suggested. The exact nature of the chemical signals associated with these processes is unknown.

The higher germination rates exhibited by polycystocarpic germlings as compared to unicystocarpic germlings seemingly correspond to a different process. It seems more likely that a larger proportion of competent spores are to be found in the polycystocarpic than in the unicystocarpic suspensions. More mature spores are probably shed first by the mature cystocarps while spore suspensions based on a single cystocarp are more likely to include younger spores as well.

Overgrowth and compression of the slower growing partner by the other germling was observed as the most probable cause of the reductions in germling survival in bispecific cultures, as compared to their respective unispecific controls. The germlings of *M. laminarioides* exhibited this effect when co-cultured with germlings of *G. chilensis,* and those of *N. fastigiata* when co-cultured with *M. laminarioides*. These culture observations also suggested that several yet poorly studied organismic factors may be most important in the outcome of these interactions. These include crust thickness, growth (expansion) rate of the growing crusts and the ways in which the crusts use the substratum. Thus, the interspecific encounter of expansive crusts (e.g. *M. laminarioides–N. fastigiata; M laminarioides–G. chilensis*) reduced germling survival of at least one of the partners. In contrast, the different use of the space by *Sarcothalia crispata*, with a narrow base and fast upright production, resulted in similar survival rates in uni- and in bispecific cultures with *M. laminarioides*. In the field, this interaction most likely is later resolved in favor of one of the two partners. However, in our laboratory culture experiments of short duration both species survived without interspecific exclusion for up to 6 months.

The number of erect axes differentiated early in development is a function of the number of spores conforming the growing sporeling (Santelices et al. 1996, 1999). Thus, if interspecific or interphase interactions reduce the number of germinating spores, such a reduction should also affect the number of axes produced by a given germling. This interpretation is consistent with some of our results in the interspecific experiments. Thus, the germlings of *M. laminarioides* and *G. chilensis* in bispecific cultures differentiated fewer erect axes than their unispecific controls, a difference consistent with the fact that in bispecific cultures both species had lower germination rates than their respective unispecific controls. In fact, the difference was statistically significant in *M. laminarioides*.

The above interpretation, however, cannot explain the lower number of erect axes differentiated by the polycystocarpic germlings of *M. laminarioides* as compared to the unicystocarpic germlings. The existence of a numerical relationship between the initial spore number and the number of erect shoots in coalescing red algae

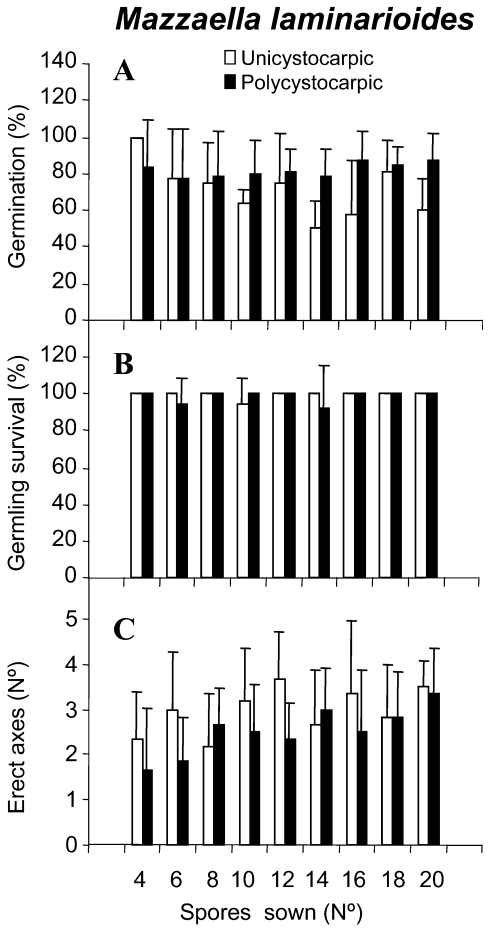

Fig. 8A–C Germination rates, sporeling survival and erect axes differentiation in uni- and polycystocarpic germlings of *M. laminarioides*. *Bars* are SE

suggests, that the clusters of spore derivatives produced by the original spores might retain their capacity to form uprights after coalescence. A lower ratio of spores to erect shoots in polycystocarpic than in unicystocarpic germlings may indicate a proportionately larger number of initial spores or derivatives unable to develop erect shoots, either by incompatibility between cells from spores derived from different cystocarps or by involvement of a proportionately larger number of cells in the polycystocarpic fusion process, not forming derivatives and, eventually, upright shoots. Additional experiments are required to explain the difference in number of erect axes, differentiated by uni- and polycystocarpic germlings. Such differences also were found in germlings of *G. chilensis* (Santelices et al. 1996) and perhaps represent a general response among coalescing Rhodophyta. However, as Gabrielson and Garbary (1986) have remarked, many important themes of vegetative development in the red algae have been overlooked over the last 30 years, including initiation of upright axes and thallus differentiation. It is therefore very difficult to advance additional explanations of the observed differences between unicystocarpic and polycystocarpic germlings.

Overall, our results suggest that red algae should be included among the several groups of sessile modular organisms that dominate marine hard-substratum habitats and are able to fuse or coalesce producing genetically composite entities (chimeras). Contrary to colonial animals and fungi, however, allorecognition seems to play a minor role in coalescence. Our results indicate that coalescence does not occur between different species but it may occur between different phases of a similar species. Thus, coalescence appears as a process less sensitive to genetic incompatibility than cell fusions, as shown in *Griffithsia pacifica* (Koslowsky and Waaland 1984, 1987), probably because it involves mostly regulation and compatibility of cell growth. Differentiation of erect axes appears as the potentially most promising response to detect signs of intraspecific incompatibility, while it remains to be seen whether secondary pit connections may be established among coalescing haploid and diploid phases of a same species.

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