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Gametophyte-sporophyte coalescence in populations of the intertidal carrageenophyte *Mazzaella laminarioides* (Rhodophyta)

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Abstract *Mazzaella laminarioide*s has consistently been reported as a typical coalescent/clump species with a triphasic life history of the *Polysiphonia*-type in which the haploid gametophyte is the predominant phase with respect to the diploid sporophyte. Preliminary observations of intertidal populations revealed that, in some instances, cystocarpic and tetrasporic fronds emerged from the same clump (G-T clumps), implying a coalescent process of haploid and diploid thalli by fusion of their corresponding adjacent basal holdfasts. Population surveys at three sites in Coliumo Bay, central Chile, were carried out to characterize frond demography as well as to asses the frequency of gametophyte-tetrasporophyte (G-T) coalescence. Visual and resorcinol methods were employed to determine the phases of the fronds collected over central transects of 15 randomly sampled clumps. Coalescence of G-T clumps was infrequent, with gametophytes dominating over tetrasporophyte thalli.

Keywords Frond demography · Gametophyte-sporophyte fusion · Gigartinaceae · Intertidal seaweed

Abbreviations

G-T	Gametophyte - Tetrasporophyte
G-G	Gametophyte - Gametophyte
T-T	Tetrasporophyte - Tetrasporophyte
G-T clumps	Gametophyte - Tetrasporophyte clumps
EBM	Marine Biological Station

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Introduction

Coalescence is a common process in which adjacent germlings fuse together; this occurs in some Rhodophyceae species as well as in other vegetable and animal organisms. The fusion is even more absolute given the connection between secondary pit connections of cells from the two adjacent germlings (Tveter-Gallagher and Mathieson 1980). This fusion results in a clumpy individual composed of more than one genet, with many fronds derived from the two original neighboring spores emerging from a common attachment holdfast. More than 30 species of red algae belonging to seven orders have been identified as having discoidal development followed by the formation of a basal crust and coalescence when two co-specific spores germinate (or two germlings grow) close enough to one another that cells of both germlings come into contact and establish secondary pit connections between them (Santelices et al. 1999). Although coalescence in seaweeds with basal disks from which erect shoots emerge was first documented by Rosenvinge (1931), intensive research on structural interconnections between different coalescent red algae, as well as the physiological and evolutional implications of such complex organisms, was only done after the Maggs and Cheney (1990) review of coalescence in several species and that of Martínez and Santelices (1992), which referred specifically to the coalescence of *Mazzaella laminarioides* (Bory de Saint Vincent). See Santelices et al. (1999) and Santelices (2004) for extensive recent reviews on coalescent macroalgae.

Mazzaella laminarioides (Bory de Saint Vincent) Fredericq is an endemic intertidal Chilean species distributed along the continental shore of Chile from Bosque Fray Jorge National Park (30° 34′ S; 61° 06′W; Vásquez and Vega 2004) to the Diego Ramírez Islands (56° 30′S; 68° 43′ W;

Mansilla and Navarro 2003). Its wide distribution indicates a great thermal tolerance; whereas the mean sea surface temperature in the northern locations fluctuates between 12°C and 17°C (winter-summer), at the Subantarctic limits of the species, it ranges from 4°C to 9°C (winter-summer), with nearly freezing air temperatures during winter low tides.

As all Gigartinaceae species, *M. laminarioides* exhibits a "*Polysiphonia* type" life history, with free-living tetrasporophyte and gametophyte phases and carposporophytes that always live in the cystocarp of the fertilized female gametophyte. Coalescence between *M. laminarioides* germlings has been demonstrated by Santelices et al. (1999, 2004) and in vitro contact between intra and interspecific haploid and diploid sporelings was reported by Santelices et al. (2003b).

Studies of *M. laminarioides* (as *Iridaea*) at Pelancura (33°35′ S; 71°38′W; Hannach and Santelices 1985) and at Matanza (33° 56′S, 71° 53′W; Santelices and Norambuena 1987) revealed a clear gametophytic dominance in the northern populations. Thornber and Gaines (2003, 2004) also found haploid dominance at 10 sites between Punta Talca (30° 57′ S; 71° 35′W) and Pichilemu (34° 24′ S; 71°59′W). Contrasting with these findings, Westermeier et al. (1987) pointed out a lack of clear dominance between both phases at the more southern site of Mehuin (39°24′S; 73′14′W).

Coalescence among nearby clumps have been studied in the field at Caleta Maitencillo (32° 30′S; 71° 29′W) and Topocalma (34° 05′S; 71° 58′W), and the results suggested that coalescence between *M. laminarioides* clumps could be a frequent process (Santelices et al. 2003a, b, 2004). Visual observations done in Coliumo Bay (H. Romo, personal observations), central Chile, showed a demographic pattern of haploid gametophytic dominance in the *M. laminarioides* population and a likely low frequency of coalescence among gametophytic and tetrasporophytic clumps (biphasic clumps). This is the first report of the frequency of coalescence between gametophytic and sporophyte thalli in the field.

Material and methods

Samples were collected at three sites along the inner shore of Coliumo Bay (36° 32′ S; 72° 56′W) in spring 2004. The sites (Fig. 1) were rocky areas at: i) Litril, with rocky platforms adjacent to an area of boulders in the innermost part of the bay; ii) Marine Biological Station (EBM) at the Universidad de Concepción, where rocky platforms are surrounded by sandy beaches, and iii) Caleta Villarrica, a beach with boulders and cobblestones (with *M. laminarioides* on top) intermingled with gravel on the east coast of the bay (Fig. 1).

Individual clumps of *M. laminarioides* were sampled haphazardly after careful visual examination to be sure of the spatial integrity and continuity of the basal disks. Twenty seven clumps were sampled at Litril and Caleta Villarrica and 30 at EBM, totaling 84 clumps for the three sites. Clump diameter of basal disks varied between 1 and 13 cm and the number of fronds sampled from each clump varied between 7 and 63 depending on the size of the clumps. Each clump was sampled by means of one transect, consisting in a 20 cm straight steel wire, placed trough the fronds, and crossing the center of the main diameter of the clump (Fig. 2). All fronds in contact with one side of the wire were detached in order; from one end of the clump to the other (Fig. 2). The fronds were strung across an iron pin in the same order that they were detached (Fig. 3). The determination of the karyological phase (haploid gametophyte or diploid tetrasporophyte) of the fronds from each clump was carried out by the resorcinol method (Garbary and De Wreede 1988). Thus, the order of the frond sequence analyzed from each pin coincided with the sampled frond sequence of each clump in the field. In the paper, clumps composed exclusively of gametophytes are identified as G-G, those consisting only of tetrasporophyte fronds as T-T, and biphasic clumps (sequences of gametophytes followed by tetrasporophyte fronds or vice-versa) as G-T (Fig. 3). In this paper, and based on its "*Polysiphonia* type" life history, we have assumed that gametophytes (resorcinol + fronds) are haploids and tetrasporophytes (resorcinol - fronds) are diploids notwithstanding nobody have elucidate the karyotype of both phases in this species.

Results

The karyological phase composition displayed by the fronds differed to some degree at the three sites. Whereas the T-T

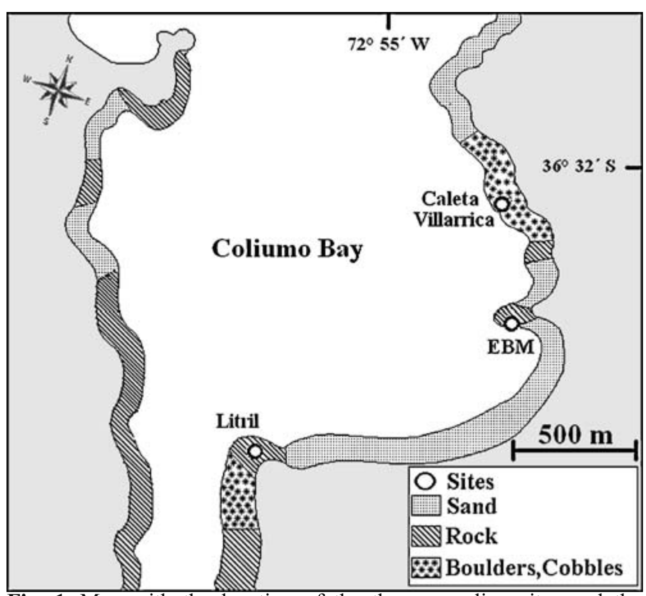

Fig. 1 Map with the location of the three sampling sites and the respective types of substrata: Litril, EBM, Caleta Villarrica

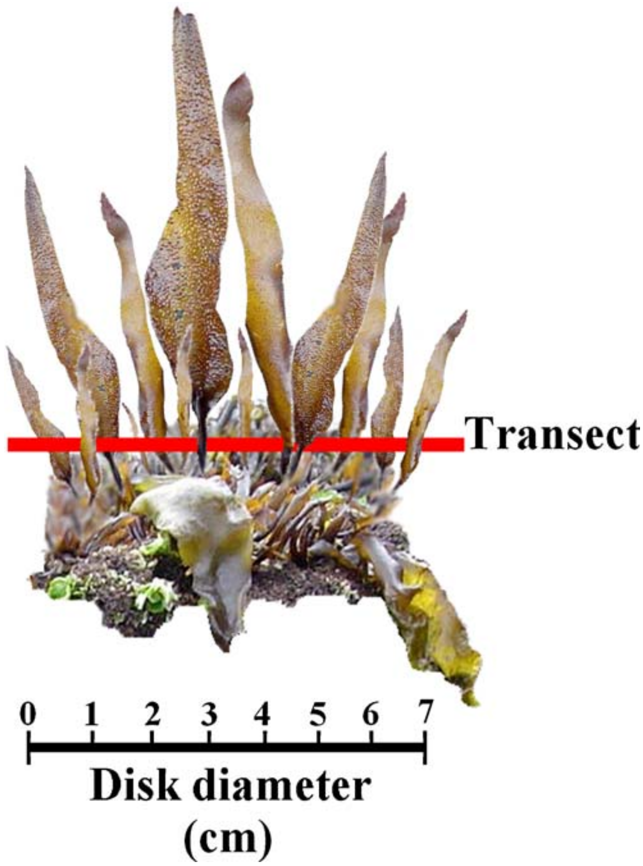

Fig. 2 Disposition of the fronds of *Mazzaella laminarioides* along transect on the basal crust

sequence was low but surprisingly identical for clumps at all sites (Table 1), the G-G sequence varied from 14 clumps at Litril to 21 at EBM. The G-T sequence was similar at Litril and EBM, but not observed at all at Villarrica (Table 1). The frond composition sequence of the pooled clumps for all three sites was 60.7% G-G, 21.4% T-T, and 17.9% G-T (Table 2), for a ratio of 6:2:2 (χ^2=0.286; 0.90<P<0.75).

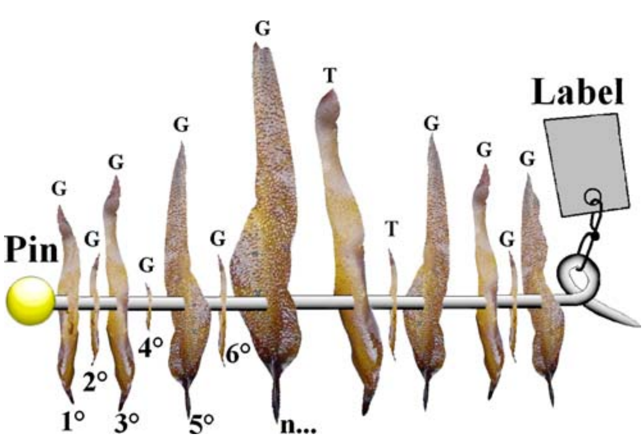

Fig. 3 Disposition of the fronds of *Mazzaella laminarioides* obtained from the basal disk in the pin

Table 1 Number of clumps and percentage of gametophytic (G-G), tetrasporophytic (T-T), and gametophytic-tetrasporophytic (G-T) frond sequences in clumps of *Mazzaella laminarioides* in Coliumo Bay

Type of clumps	Litril	EBM	C. Villarrica	Total	%
G-G	14	16	21	51	60.7
T-T	6	6	6	18	21.4
G-T	7	8	0	15	17.9
Total clumps	27	30	27	84	

Clumps with biphasic sequences were found only at Litril and EBM, and most of these samples had a pattern of the type: G-G-...-G-T-T-...-T or, inversely, T-T-...-T-G-G-...-G, depending on the end of the clump diameter where we began the sampling. Only one sample from EBM and two from Litril had mixed biphasic sequences of the G-G-T-G-...-G-T-T-T type, that is, at least one tetrasporophyte (or gametophyte) frond was found among a series of fronds of the opposite phase. Clumps having both mature cystocarpic and tetrasporic fronds were not frequent during the study period (spring 2004); only one such clump was observed at Litril and two at EBM (3.6% of all 84 clumps analyzed).

The analysis of the proportions of gametophytic and tetrasporophytic fronds showed a general strong dominance (71.5%) of haploids (Table 2); this did not differ significantly from the 76% reported by Thornber and Gaines (2004) for northern sites (χ^2=0.666; 0.75>P>0.50). In the three stands, the G: T ratios were always greater than 1 (Table 2), reaching 3.66 at Caleta Villarrica and 2.50 for the pooled data from all three sites.

Discussion

This research confirmed the preliminary assumption about natural field coalescence between karyologically different individuals of *M. laminarioides*, which was based on preliminary field observations of clumps that exhibit both cystocarpic and tetrasporic fronds (Fig. 4).

Of the 84 clumps analyzed, the G-T frond sequences reached 17.9%, similar to the 21.4% observed for T-T sequences; the statistical similarity between these two

Table 2 Number of fronds of each phase and G: T ratio of *Mazzaella laminarioides* clumps in the sampled transects in Coliumo Bay

Type of fronds	Litril	EBM	C. Villarrica	Total	%
Gametophyte	208	306	315	829	71.5
Tetrasporophyte	111	134	86	331	28.5
Total fronds	319	440	401	1160	100
G : T ratio	1.87	2.28	3.66	2.50	

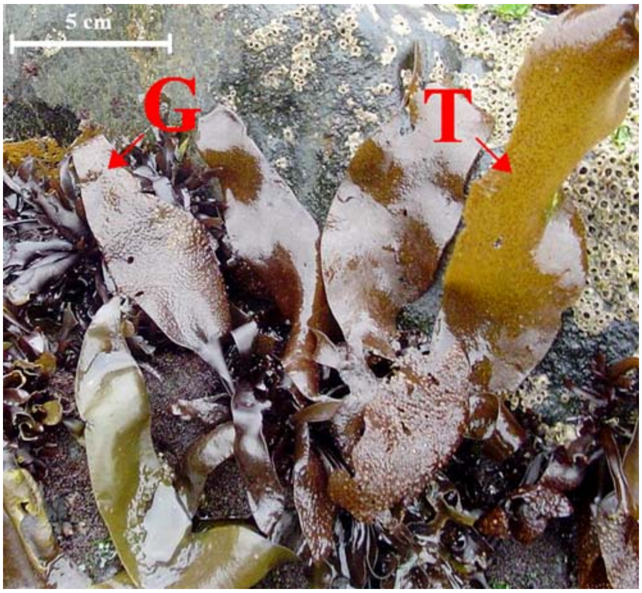

Fig. 4 Coalescent clump in the field showing a mature cistocarpic frond (G) and a tetrasporic (T) one

sequences suggest a very close relationship between G-T coalescence and the amount of diploid tetrasporophytic fronds at nearby sites. However, no reasonable explanation was found for the absence of G-T clumps at Caleta Villarrica. The G-T frequency at this site could have been so low that it was not detected by the sampling, although there were no obvious causal factors - biological or environmental - explaining the G-T absence. The most visible attributes of the shore at Caleta Villarrica are its unstable, rocky substrate and the very small basal disk of clumps growing on top of the boulders (up to 5 cm diameter in our sampling). The site is composed mainly of boulders and stones intermingled with coarse sand and gravel. Periodically, strong winter storms (3–4 per year) create surges between the boulders. The possible interference on coalescence by frequent collisions by stones on boulders during storms could be producing more frequent disruption of basal disks. This fact could be the explanation for the absence or low frequency of G-T sequences and the small size of the disks, but this study does not have available data to sustain this hypothesis. The harvest pressure is rather similar for the three sites because the population of hand harvesters that operate during spring low tides is the same and no great differential pressure seems to exist between sites. A survey of the interaction between substrate stability/instability and basal disk size at sites with and without unstable rocky substrata could be one way to help decipher this result. The G-T coalescence pattern between karyologically different *M. laminarioides* partners mostly showed fronds with G-G-G-G-...-T-T-T type arrangements and, less frequently, G-G-G-**T**-G-...T-T. In the first case, coalescence was the product of two karyologically different spores (or two karyologically different group of spores) that initially germinated and grew as two spatially segregated groups. The growth was such that, after the fusion of their basal disks, one or several tetraspores developed G-G-G-G... sequence of fronds in one side of the crust, whereas the other side of the disk was the result of the germination of one or several carpospores giving a...T-T-T sequence of fronds. In this case a natural bifacial chimera with two different karyologies occurs. In the first case, clumps composed of disorderly G-T frond sequences (*e.g.* G-G-G-**T**-G-...T-T) constituted a multifacial chimera, also with fronds of different karyologies, but generated by groups of carpospores and tetraspores that germinated spatially intermingled.

This is a general case of chimerism in *M. laminarioides* but a more subtle one was reported by Santelices (2004, see Fig. 5 of cited paper) using PCR RAPD analysis in a *M. laminarioides* gametophyte clump. In that study, three genetically different kinds of fronds were detected when eight haploid fronds were sampled from the same clump. The same chimerical process could be occurring in both T-T and G-T clumps.

On the other hand, the low observed frequency of biphasic clumps reflected an inequality of phases such that, at least in its northern distribution, the species is always strongly gametophyte dominant (Santelices and Norambuena 1987; Thornber and Gaines 2003, 2004). Variations in the proportion of phases may be found in the more southern populations, as was observed at Mehuin, Valdivia Province (39° 24′S; 73° 14′W), approximately 300 km south of Coliumo Bay, where Westermeier et al. (1987) found no differences between phase proportions. Moreover, the species' demography is absolutely unknown along the 1350 km from Mehuin to the Diego Ramírez Islands (56° 30′S; 68° 43′), the site reported as the southern distributional limit (Mansilla and Navarro 2003). In order to determine the homogeneity or heterogeneity of the population along the species' distributional range, further field studies on the proportion of phases and intra and interphase frequency of coalescence are required in the Subantarctic areas. Here, the species lives in cold environmental conditions that come close to freezing during winter low tides, receiving higher UV radiation and suffering extreme winter-summer photoperiods.

No available data on the temporal variation of G-T coalescence exists, but it can be assumed as a hypothesis that due the perennial quality of the basal disks no great changes could be at least expected in small temporal scale.

As an overall conclusion, in these kinds of clumpy algae, the concept of a thallus as an individual composed of an encrusting holdfast with many fronds of the same origin is clearly misleading and can not be applied to coalescent species because a clump is not always a genetically homogeneous individual.

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