J. Phycol. **37,** 705–716 (2001)

HIERARCHICAL SPATIAL STRUCTURE AND DISCRIMINANT ANALYSIS OF GENETIC DIVERSITY IN THE RED ALGA *MAZZAELLA LAMINARIOIDES* (GIGARTINALES, RHODOPHYTA)[1]

Sylvain Faugeron

Departamento de Ecología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Casilla 114-D, Santiago, Chile and Laboratoire de Génétique et Evolution des Populations Végétales, UPRESA CNRS 8016, GDR 1002, Université de Lille I, Bât. SN2. F-59655 Villeneuve d'Ascq cedex, France

Myriam Valero,[2] *Christophe Destombe*

Laboratoire de Génétique et Evolution des Populations Végétales, UPRESA CNRS 8016, GDR 1002, Université de Lille I, Bât. SN2. F-59655 Villeneuve d'Ascq cedex, France

Enrique A. Martínez and Juan A. Correa

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

Our study of the genetic structure of *Mazzaella laminarioides* (Bory) Fredericq (Gigartinales) in the central Chilean region documented a high level of genetic diversity based on random amplified polymorphic DNA (RAPD) markers and indicated the occurrence of significant genetic structure at different spatial scales. A total of 288 haploid gametophytes was analyzed with 17 polymorphic RAPD bands, which produced 202 distinct multilocus genotypes. Within stands, mean gene diversity ranged from 0.210 to 0.249 and no significant linkage disequilibrium could be detected among pairs of alleles, revealing that recombination (sexual reproduction) regularly shuffles the genes at that scale. Analysis of molecular variance within stands (less than 30 m) showed that the structure was very low, only marginally significant, and did not increase with increasing hierarchical levels at this lowest spatial scale. In contrast, at a larger spatial scale (among stands, from 5 to 60 km), increasing geographical distance seemed to account for increasing isolation between populations even if natural barriers, such as sandy beaches or river estuaries, may play a role in such isolation. Moreover, the strong genetic differentiation occurring between locations separated by 60 km allowed the assignment of individuals to their original population through a canonical discriminant analysis. This approach further allowed the identification of potential recent migrants from one population to the other. Thus, in species like *M. laminarioides* for which the dominance of RAPD markers can be avoided by selecting haploid individuals, RAPD analysis appeared to be specially appropriate for the study of genetic differentiation.

Key index words: **assignment test; seaweed dispersal; gene flow; *Mazzaella laminarioides*; population genetic structure; RAPD**

Abbreviations: **RAPD, random amplified polymorphic DNA; AMOVA, analysis of molecular variance**

Population genetic structure is a critical feature of a species because of its potential effect on rates of evolutionary change. Population genetic theory predicts that even quite low rates of migration are "evolutionary significant" in that they can (depending on the balance between selection and migration) prevent adaptation to local conditions and, ultimately, speciation (for review see Slatkin 1987). The habitat of most species of plants and animals is discontinuous, resulting in an aggregated distribution of individuals into local populations. If these local populations are sufficiently small and isolated, the effect of genetic drift may become the primary evolutionary force that will favor their genetic divergence.

Neutral molecular markers are very powerful in exploring the existence of genetic differentiation (Sunnucks 2000). In marine organisms characterized by limited dispersal capabilities such as sessile invertebrates (Knowlton and Jackson 1993), reef fishes (Shulman 1998, Riginos and Nachman 2001), sea grasses (Procaccini and Mazzella 1998, Ruckelshaus 1998), and seaweeds (for review see Sosa and Lindstrom 1999), significant genetic differentiation at various spatial scales has been reported. Bohonak (1999) pointed out that in marine species, the apparent continuity of dispersal (through a pelagic phase) can be limited by physical, chemical, and biological barriers, thus limiting gene flow among populations. For example, Riginos and Nachman (2001) showed that sandy beaches or open water masses could interrupt the gene flow usually occurring over a continuous suitable habitat (the rocky shore). Furthermore, recent studies on the pattern of genetic substructure occurring over very small scales (<10 m) in several marine coastal organisms, including invertebrates (Yund and O'Neil 2000), sea grasses (Ruckelshaus 1998), and seaweeds (Williams and DiFiori 1996, Wright et al.

[1]Received 16 April 2001. Accepted 3 July 2001.

[2]Author for correspondence: e-mail myriam.valero@univ-lille1.fr.

2000, Zuccarello et al. 2001), revealed that populations are often subdivided into different breeding units (but see McFadden and Aydin 1996). The spatial scales over which a population differs genetically are readily accessible using a hierarchical sampling design. On a local scale, the life span of spores and gametes after being released determines the dispersal range (movement of genes within populations) of benthic seaweeds. At the scale of a few meters, significant clusters of related genotypes occurred within distances of 2–3 m in the hermaphroditic fucalean *Silvetia compressa* (as *Pelvetia fastigiata*, Williams and DiFiori 1996) for which limited dispersal of zygotes was suggested by Johnson and Brawley (1998). On the other hand, spatial autocorrelation analyses did not reveal any significant structure in the fucalean *Halidrys dioica*, a species characterized by floating (detached) reproductive fronds (Lu and Williams 1994). For many Rhodophyta, dispersal of spores and spermatia is likely to be limited due to their short life span, absence of flagella, and high sinking rates (Santelices 1990). Recently, movement of male gametes was traced within a natural population of *Gracilaria gracilis* using microsatellite loci, revealing a large excess of matings at short distances (less than 1 m from the paternal thallus, Engel et al. 1999). On a larger scale, mechanisms for long distance dispersal, such as floating fronds or thallus fragmentation, in addition to spore and gamete dispersal can occasionally reinforce gene flow between populations (Lu and Williams 1994).

Mazzaella laminarioides (Bory) Fredericq (Gigartinales) is an ecologically and economically important species in central and southern Chile. Restricted to rocky beaches, it forms extended stands in the mid and low intertidal zones (Santelices 1991). Despite its importance, there is no information on the dynamics of *M. laminarioides* populations or on how much they are interconnected through gene flow. Some biological characteristics may suggest that dispersal ability in *M. laminarioides* is limited. First, Hoffmann and Camus (1989) demonstrated that spores generally settle rapidly after release. Second, dispersal by thallus fragments has never been reported in this species. Due to the lack of floating structures, broken thalli sink and decompose rapidly and are thus unlikely to disperse over long distances. These elements raise the question of the potential effect of limited spore dispersal on the genetic diversity of wild stands of this species. Stands of *M. laminarioides* form long stretches of up to several kilometers that are spatially separated by long sandy beaches and estuaries along the Chilean coast. Within stands, distribution of *M. laminarioides* individuals is continuous, except for small and irregular interruptions due to the topography of the substratum, inherent to rocky intertidal shores. Taking into account that there is no precise estimate of the effective dispersal distances of male gametes and spores, it is difficult to make any prediction on the pattern of distribution of the genetic diversity within a stand. However, several field studies revealed a patchy occurrence of individuals infected by the cyanobacterium *Pleurocapsa* sp. This pathogen causes the development of tumors and severe frond deformation (Correa et al. 1993) and decreases the reproductive potential of the host (Faugeron et al. 2000). The full expression of the disease occurs in discrete areas, sharply delimited from the remaining healthy stand, where most of the host individuals are severely diseased (Correa and Sánchez 1996, Correa et al. 1997). This aggregated distribution of infected individuals either indicates that the abiotic environment is sufficiently heterogeneous at small spatial scales to permit a patchy pattern of disease expression or that the biotic environment of *Pleurocapsa* sp. (i.e. the host population) is heterogeneous at scales of a few meters. In this latter context, significant spatial substructuring within stands of the *M. laminarioides* would be expected.

Although allozymes have been successfully used to examine genetic structure for many organisms, they are generally not ideal tools in seaweeds because of the low number of loci available and their low level of polymorphism (Sosa and Lindstrom 1999, Valero et al. 2001). In this context, a diverse array of molecular tools has been developed in several algal species for high-resolution population-level genetic studies, including microsatellites in the red seaweed *Gracilaria gracilis* (Luo et al. 1999) and in the kelp *Laminaria digitata* (Billot et al. 1998), amplified fragment length polymorphisms in the kelp *Alaria marginata* (Kusumo and Druel 2000), and random amplified polymorphic DNA (RAPD) in several red algal species (Wright et al. 2000, Meneses 2001) and several kelp species (Coyer et al. 1997, Miller et al. 2000). RAPDs (Welsh and McClelland 1990, Williams et al. 1990) are widely used in determination of population genetic structure because they are not technically demanding. This method simultaneously amplifies many regions of genomic DNA and therefore provides virtually unlimited number of loci. Furthermore, this attribute avoids the potential problem of sampling loci that, having a common evolutionary history, could produce different polymorphism patterns (Sunnucks 2000).

In the present study, we examine the patterns of genetic structure in the red alga *M. laminarioides* at five spatial scales (from less than 5 m to 60 km). We used 17 RAPD loci, detected by four random primers, to analyze the pattern of genetic structure among and within four natural stands of *M. laminarioides*.

MATERIALS AND METHODS

Study species. Individuals of *M. laminarioides* produce deciduous laminar fronds from a perennial holdfast attached to the substratum. This species is characterized by a typical *Polysiphonia*-type life cycle, with coexisting and isomorphic tetrasporophytes (diploid) and gametophytes (haploid).

Sampling design. To assess the pattern of genetic structure occurring within and among natural beds of *M. laminarioides*, a hierarchical sampling design was implemented. Five spatial levels were considered: 1) locations separated by 60 km, 2) stands separated by 5 km, 3) plots separated by 25 to 30 m, 4) quadrats sampled 5 m apart, and 5) individuals within 1-m^2 quadrats (Fig. 1). Two locations were included in the study: Santo Do-

mingo (STDO; 33°37′S, 71°40′W) and Topocalma (TOPO; 34°05′S, 71°58′W), both located in central Chile (Fig. 1). Within Topocalma, three stands separated by sandy beaches of about 5 km were studied (TOPO South, TOPO Middle, and TOPO North, Fig. 1). Within each stand, four quadrats (A, B, C, D) grouped into two plots (A, B vs. C, D) were sampled. Thus, 12 quadrats in all were defined within the Topocalma location. The same design, with only one stand, was repeated within Santo Domingo (Fig. 1). Within each location, one of the sampled quadrats (i.e. TOPO Middle-A and STDO-A) was located in a patch of plants severely infected by *Pleurocapsa sp.* Approximately 40 juvenile and healthy fronds of *M. laminarioides* were collected in each of the 16 quadrats. Only one frond, and thus only one single genotype, was collected from each individual holdfast. To avoid sampling fronds of the same holdfast, fronds were collected at least 10 cm apart from each other. All collected fronds were stored in plastic bags, kept refrigerated (4–8° C), and taken to the laboratory where haploid gametophytes were sorted using the resorcinol test (Craigie and Leigh 1978). Only gametophytic individuals were genotyped (see below), and consequently the resulting sample size was between 15 and 22 individuals per quadrats. The selected haploid fronds were carefully washed in fresh water, sonicated, brushed, and rinsed in distilled water. Areas of the fronds with signs of infection, necrosis, abnormal pigmentation, or presenting any evidence of potential contamination by other organisms (checked under stereoscopic microscope when necessary) were removed, and the resulting clean fragments were air dried overnight at 40° C and kept frozen at −20° C until DNA extraction.

DNA extraction. DNA was extracted from tissue finely ground in liquid nitrogen, following a protocol slightly modified from that described by Saunders (1993). Approximately 10–20 mg of dry tissue was incubated at room temperature for 1 h in 800 μL of lysis buffer (0.1 M Tris base, pH 8.0, 0.05 M EDTA, 0.2 M NaCl, 2.5 M KAc) plus 100 μL of Tween 20 (10%) and 8 μL of proteinase K (20 mg·mL^{-1}). Purification of the DNA solution consisted of adding 700 μL of phenol:chlorophorm:isoamyl alcohol (25:24:1) directly to the lysis buffer and homogenizing by inversion during at least 3 min before centrifugation for 20 min at 22,000*g* and −5° C. The aqueous phase was extracted a second time by one volume chlorophorm:isoamyl alcohol (24:1) and centrifuged for 3 min. DNA was precipitated by 0.6 volume of iso-propanol and pelleted by 15 min centrifugation at −5° C and 15,000*g*. The pellet was washed with 70% ethanol, vacuum dried, and resuspended in 200 μL of TE buffer (Tris HCl 10 mM, EDTA 2 mM, pH 8.0). DNA quality and quantity were estimated by electrophoresis in agar gel and UV absorption, respectively, using a SmartSpec spectrophotometer (Bio-Rad, Hercules, CA) at 260 and 280 nm.

PCR amplification. PCRs were performed in a Perkin Elmer GenAmp 9700 Thermocycler, using 2–3 ng of template DNA, in a 25 μL mix containing 1 unit of GIBCO Brl Taq polymerase, 2 μL of 10 Buffer, 2.2 μL $MgCl_2$ (50 mM), 30 pmol of primer (Genset Oligos, San Diego, CA), and 1.6 μL dNTP mix (Gibco BRL, Rockville, MD) (at 2.5 mM each). Primers were selected from a set of 44 primers according to their polymorphism and reproducible banding patterns tested on subsamples of a few individuals coming from two localities separated by 1000 km. Four primers were retained for the present study: A2 (5′-TGC CGAGCTG-3′), X7 (5′-GAGCGAGGCT-3′), X12 (5′-TCGCCAG CCA-3′), and X17 (5′-GACACGGAAC-3′). Amplified DNA fragments were separated by electrophoresis on a 1.5% TAE agarose gel stained with EtBr. Reproducibility was maximized by strictly following the recommendations summarized by Harris (1999). To ensure homology in band size among samples from distinct locations, PCR and electrophoresis gel were always run with a mixture of samples from various geographical origins. Bands were scored manually from printed pictures. Only products of a limited size range (300–2500 base pairs and including monomorphic bands) were scored so that the quality of RAPD amplification could be ascertained. Only bands of high intensity characterized by a clear-cut pattern of presence/absence were retained for the analysis, and samples that did not present reproducible banding patterns were systematically discarded from the analysis. Each PCR included a negative control made of the same sterile deionized water used to dilute DNA samples. To ensure that the detected genetic variability was entirely attributable to differences between *M. laminarioides* individuals and not to the endophyte *Pleurocapsa*, individuals collected in infected and noninfected quadrats were compared.

Only haploid individuals were used to facilitate analysis: each polymorphic band was considered as a locus with two alleles ("present" and "absent"), and the allelic frequencies could be directly determined without any correction for dominance. Because *M. laminarioides* is a coalescing species (Santelices et al. 1999), the possibility exists that chimeric fronds might develop, as described for *Gracilaria chilensis* (Santelices et al. 1996). Such organisms would carry two or more different genotypes (Santelices et al. 1999), unlikely detectable by a dominant marker such as RAPD. In *M. laminarioides*, however, all the experimental evidence accumulated so far indicates that each original cellular lineage in coalescing individuals retain, at least for the first emerging axes, the ability to generate its own meristem. Therefore, in our study, each sampled frond was considered to

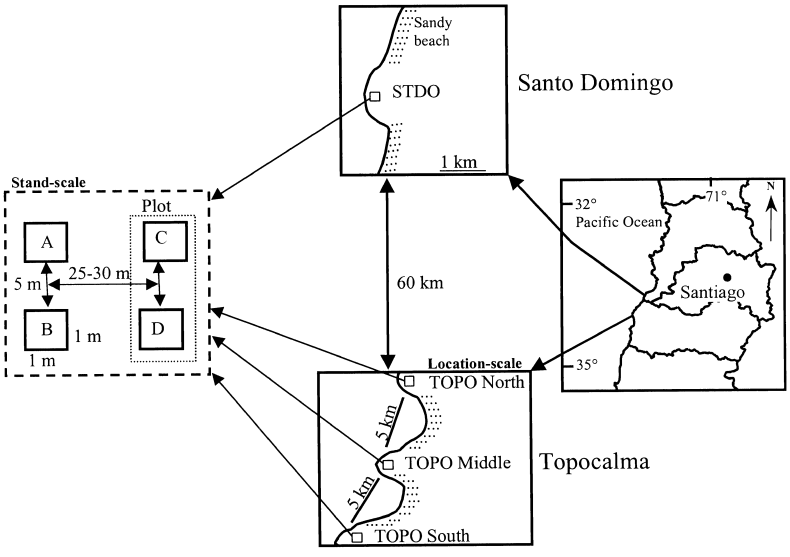

FIG. 1. Hierarchical sampling design used for collecting *Mazzaella laminarioides* individuals on the coast of central Chile.

be the expression of a single haploid genotype. Three types of data analyses were performed. The first considered each locus separately and used classical gene frequencies (monolocus data analysis). The second analysis treated the individual banding profile as a multilocus genotype and used genotype frequencies (multilocus data analysis). Finally, the data were also analyzed using a multivariate approach (linear discriminant analysis).

Monolocus data analysis. For each quadrat and stand, allele frequency and gene diversity (measured as the expected heterozygosity H averaged over loci [Nei 1978]) were calculated using the GENETIX package version 4.0 (Belkir et al. 1996). For each RAPD band, the gene diversity averaged over the four stands was estimated using the FSTAT version 2.9 program (Goudet 1999).

To test whether the different bands were randomly associated or linked within banding profiles, gametic linkage disequilibria (Lewontin and Kojima 1960) between RAPD loci were estimated using the software ARLEQUIN (Schneider et al. 2000). These disequilibria may be due to physical linkage on a chromosome, to a lack of recombination caused by clonal propagation (selfing), or to differences in allele frequency among populations (spatial genetic structure). Physical linkage implies that the same allele pairs will be systematically observed over all spatial scales, whereas in the two other cases, linkage disequilibria do not generally imply the same allele pairs. Finally, if due to spatial structure, linkage disequilibria should vary with the spatial scales. For each pair of loci and each of the four *M. laminarioides* stands and in the overall sample, linkage disequilibria were tested by Fisher exact tests. Because multiple tests were conducted, a Bonferroni sequential procedure was used to correct significance levels (Rice 1989).

Because the sampling was hierarchical, gene frequencies were analyzed using a nested analysis of variance (AMOVA, analysis of molecular variance, Excoffier et al. 1992). AMOVA estimates the components of variance of allele frequencies and Φ-statistics analogues of F statistics (see Excoffier et al. 1992). Considering a group consisting of several units subdivided into different subunits, the principle of AMOVA is to partition the total genetic variation observed among subunits into an among-units component and an among-subunits-within-units component. The classical measure of genetic differentiation among all subunits F_{ST} (or its analogue Φ_{ST}) is partitioned into Φ_{CT} (a measure of genetic differentiation among units) and Φ_{SC} (among subunits within units). Five separate analyses were conducted. First, variation at the scale of stand (hereafter stand-scale analysis) was examined by determining the variance component attributable to among-plots and to among-quadrats within plots. This analysis was performed separately within each of the four stands containing two plots and four quadrats. Second, variation at the scale of location (hereafter location-scale analysis) was examined by determining the variance component attributable to between-stands and to between-quadrats within stands. The location-scale analysis was only conducted within the Topocalma location, which included three different stands. The significance of the Φ statistics was tested using 100,000 permutations (permuting individuals among subunits among units for Φ_{ST}, permuting individuals among subunits within units for Φ_{SC}, and permuting subunits among units for Φ_{CT}). Finally, the differentiation between the two study locations was assessed by averaging the three F_{ST} values calculated between Santo Domingo and each of the three stands of Topocalma (multilocus F_{ST} estimated with FSTAT).

Multilocus data analysis. Examining multilocus genotype diversity can assess the importance of recombination. In a species reproducing exclusively asexually, genotype diversity is the result of the combined effects of mutation and migration, whereas in an outcrossing species recombination is the principal source of diversity. The number and frequency of shared versus different multilocus genotypes were then estimated at each spatial scale using ARLEQUIN (Schneider et al. 2000).

Discriminant analysis. To test the power of the RAPD loci to discriminate among stands or locations, a linear discriminant analysis was performed with the software STATISTICA (StatSoft, 1995) using each locus as a predictor variable (with two states: presence or absence of the fragment) and stands as the group classification variable. The group of origin (i.e. stands) can be predicted for each individual by a set of discriminating functions (the maximum number of discriminating functions corresponding to $n - 1$ groups). In the case of high discrimination (i.e. high genetic differentiation among stands), this method allows identification of putative migration events (i.e. misclassified individuals). This analysis tests the genetic differences among groups and the contribution of each locus to these differences. The contribution of each locus is evaluated by the absolute magnitude of standardized discriminant coefficients.

RESULTS

Interpretation of the banding patterns and pairwise linkage disequilibrium. Using four primers, 21 perfectly reproducible bands were scored, of which 17 were polymorphic over the 288 *M. laminarioides* individuals retained for analysis. Loci were identified by indicating first the name of the primer and then the size of the RAPD fragment (in base pairs). The number of individuals analyzed per quadrat ranged from 15 to 22.

Within stands, none of the pairwise tests of linkage disequilibria were significant after Bonferroni's correction for multiple comparisons (Table 1), suggesting that recombination regularly shuffles the alleles at different loci at this scale. This result indicates no physical linkage between any pair of loci and that each band can thus be considered as an independent locus. On the other hand, linkage disequilibria varied with the spatial scale. When tests were performed on the complete data set regrouping the two localities, ca. 20% of pairwise comparisons showed nonrandom association of alleles (Table 1). After Bonferroni's correction, five pairs of loci (i.e. 4%) still showed highly significant linkage disequilibria (Table 1), suggesting that genetic variability was structured between locations.

Genetic diversity. The comparison of allele frequencies between infected and noninfected quadrats (STDO-A vs. STDO-NI and TOPO Middle vs. TOPO Middle-NI, Table 2) showed that none of the scored bands were correlated to the occurrence of the cyanobacterium. Thus, the RAPD banding patterns were entirely attributable to the *M. laminarioides* genome, and all the analyzed loci could be considered as neutral. The level of polymorphism was highly variable among loci (Table 2). The mean gene diversity (H averaged over stands, Table 2) ranged from 0.03 for locus X12-1100 (the same allele was nearly fixed in all the study sites) to

TABLE 1. Percentage of pairwise comparisons showing significant linkage disequilibrium (Fisher exact test) within each stand of *Mazzaella laminarioides* and within the overall sample.

Sites	Number of comparisons	% significant ($P < 0.05$)	% significant after Bonferroni correction
Santo Domingo	78	12.8	0
Topocalma-North	105	0	0
Topocalma-Midle	105	3.8	0
Topocalma-South	105	0.9	0
Overall sample	120	19.2	4.2

TABLE 2. Gene frequency and gene diversity (*H*, unbiased genetic diversity, Nei 1978) for each of the 17 RAPD loci.

	Infected vs. noninfected				Stands				
	Santo Domingo		Topocalma Middle						
Loci	Infected ($n = 16$)	Noninfected[a] ($n = 16$[b])	Infected ($n = 17$)	Noninfected[a] ($n = 19$[b])	STDO ($n = 64$)	TOPO-North ($n = 67$)	TOPO-Middle ($n = 76$)	TOPO-South ($n = 81$)	*H* averaged over stands
X7-1360	0.000	0.000	0.412	0.204	0.000	0.209	0.250	0.161	0.247
X7-1310	1.000	1.000	1.000	0.965	1.000	0.910	0.974	0.988	0.061
X7-1110	1.000	1.000	0.824	0.832	1.000	0.896	0.829	0.914	0.159
X7-570	0.563	0.667	0.000	0.000	0.641	0.015	0.000	0.037	0.142
X12-1250	0.938	0.917	1.000	0.949	0.922	0.985	0.961	0.951	0.087
X12-1100	1.000	0.917	1.000	1.000	0.938	1.000	1.000	1.000	0.030
X12-820	1.000	0.896	1.000	0.983	0.922	0.985	0.987	1.000	0.051
X12-290	0.000	0.000	0.412	0.139	0.000	0.209	0.197	0.469	0.290
X17-2025	0.438	0.229	0.647	0.509	0.281	0.119	0.595	0.247	0.376
X17-1750	1.000	0.792	0.471	0.338	0.844	0.687	0.368	0.531	0.420
X17-1400	0.188	0.583	0.471	0.678	0.484	0.358	0.632	0.284	0.464
X17-1155	0.625	0.438	0.882	0.949	0.484	1.000	0.934	0.975	0.170
X17-1125	0.625	0.646	0.471	0.457	0.641	0.746	0.461	0.556	0.464
X17-700	0.938	0.917	0.882	0.797	0.922	0.582	0.816	0.543	0.362
A2-1025	0.125	0.083	0.176	0.033	0.094	0.075	0.066	0.037	0.127
A2-900	0.938	0.917	0.353	0.422	0.922	0.224	0.408	0.407	0.370
A2-740	1.000	0.938	0.882	0.949	0.953	0.970	0.934	0.901	0.114
H averaged over loci	0.170	0.210	0.278	0.229	0.210	0.214	0.249	0.246	

The frequency of "present" allele is given. Observed gene frequencies within the quadrats localized in the infected and adjacent noninfected zones, and within each stand are shown.

[a] Gene frequency averaged over the three noninfected quadrats adjacent to the infected one in each stand.

[b] Mean number of individuals over the three noninfected quadrats.

0.464 for X17-1400 and X17-1125 (i.e. almost the maximum value, $H = 0.5$, for a locus with two alleles). On the contrary, *H* was very similar from one stand to the other ranging from 0.210 to 0.249 (Table 2). Whatever the stand, 4 of the 17 loci (X7-1310, X12-1250, X12-1100, and X12–820) consistently showed a very low level of polymorphism ($H < 0.1$, Table 2).

Marked differences in allele frequencies were observed between the two locations separated by 60 km (locus X7-1360, X7-1110, X12–290, X17-1155, and A2-900, Table 2) and even among stands within location (among the three Topocalma stands, see loci X17–2025, X17-17502, X17-1400, X17-1125, and X17-700, Table 2).

Hierarchical spatial structure. The stand-scale AMOVAs revealed that more than 95% of the genetic variance was found within quadrats (Table 3A). The two spatial units (quadrats and plots) accounted for very little (less than 5%) of the total genetic variance observed within each of the four stands of *M. laminarioides* (Table 3A). At this scale, the two variance components, among-plots and among-quadrats within plots, and their associated fixation indices (Φ_{CT} and Φ_{SC} respectively, Table 3B) were not significant except for Φ_{SC} within Topocalma South (Table 3B). Nevertheless, within three of the four stands, genetic differentiation among quadrats ranged from significant ($\Phi_{ST} = 0.05$, $P < 0.01$, in Topocalma South, Table 3B) to only marginally significant (Φ_{ST} varied from 0.02 to 0.04, $P \simeq 0.05$ in Santo Domingo and Topocalma Middle, Table 3B). At this scale, differentiation did not increase with increasing spatial unit.

At the location scale, the hierarchical units (stands, quadrats) explained a significant proportion of the total genetic variance and accounted for twice the variation (9%) of the stand-scale analysis (Table 3A). Global genetic differentiation among quadrats was highly significant ($\Phi_{ST} = 0.09$, $P < 10^{-5}$, Table 3B). In addition, the two variance components, among-quadrats within stands and among stands, and their related fixation indices ($\Phi_{SC} = 0.025$ and $\Phi_{CT} = 0.07$, respectively, Table 3B) were significant. Consequently, the magnitude of the genetic differentiation measured among stands separated by 5 to 10 km is at least 2.5 times higher than that among quadrats separated by 30 m or less.

Finally, the highest F_{ST} values were observed at the largest spatial scale. The three F_{ST} calculated between Santo Domingo and each of Topocalma stand varied between 0.250 and 0.279, indicating that genetic differentiation between the two locations was 10 times higher than among quadrats within stands and highly significant ($P < 0.0001$ after 15,000 randomizations of genotypes among samples).

Multilocus genotype diversity. A total of 202 different genotypes was obtained from the 288 analyzed individuals. The distribution of the number of individuals per genotype showed that 153 individuals had a unique multilocus genotype, whereas up to 7 individuals shared a same genotype (Fig. 2). The detailed analysis (Table 4) showed that genotype diversity reached its maximum value within quadrats; thus, at the lowest hierarchical level, the number of multilocus genotypes is often very close to the number of individuals analyzed. Out of the 64–81 individuals per stand, 42–67 multilocus genotypes were recorded, indicating that only 8–11 (5%–11%) genotypes were shared

TABLE 3. Hierarchical analysis of variance of allele frequencies (AMOVA) in *Mazzaella laminarioides* at different spatial scales. (A) Variance components and percentage of variation (df, degrees of freedom; SS, sum of squares).

Source of variation	df	SS	Variance components	% total variation
Stand-scale analysis				
Santo Domingo				
Among plots within stand	1	4.02	0.065	4
Among quadrats within plots	2	3.84	0.010	0
Within quadrats	60	105.44	1.757	96
Topocalma North				
Among plots within stand	1	1.94	0.008	0
Among quadrats within plots	2	3.32	0	0
Within quadrats	63	115.64	1.835	100
Topocalma Middle				
Among plots within stand	1	2.97	0	0
Among quadrats within plots	2	6.13	0.513	2
Within quadrats	72	150.70	2.093	98
Topocalma South				
Among plots within stand	1	4.28	0.010	1
Among quadrats within plots	2	7.76	0.091	4
Within quadrats	77	156.54	2.033	95
Location scale analysis				
Topocalma				
Among stands	2	28.5	0.152	7
Among quadrats within stands	9	26.4	0.050	2
Within quadrats	212	422.9	1.995	91

(B) Fixation indices.

	Fixation indices	*P* value
Stand-scale analysis		
Santo Domingo		
Among quadrats within stand (Φ_{ST})	0.041	0.055
Among quadrats within plots (Φ_{SC})	0.006	0.362
Among plots within stand (Φ_{CT})	0.036	0.332
Topocama North		
Among quadrats within stand (Φ_{ST})	−0.001	0.534
Among quadrats within plots (Φ_{SC})	−0.006	0.584
Among plots within stand (Φ_{CT})	0.005	0.333
Topocama Middle		
Among quadrats within stand (Φ_{ST})	0.023	0.054
Among quadrats within plots (Φ_{SC})	0.024	0.090
Among plots within stand (Φ_{CT})	−0.001	0.666
Topocama South		
Among quadrats within stand (Φ_{ST})	0.047	<0.01
Among quadrats within plots (Φ_{SC})	0.043	<0.01
Among plots within stand (Φ_{CT})	0.005	0.334
Location-scale analysis		
Topocalma		
Among quadrats within location (Φ_{ST})	0.092	$<10^{-5}$
Among quadrats within stands (Φ_{SC})	0.025	<0.01
Among stands within location (Φ_{CT})	0.069	<0.001

among quadrats (Table 4A), whereas 15% of the genotypes were shared among stands in Topocalma. Only two multilocus genotypes were shared between the two locations, Santo Domingo and Topocalma (Table 4B).

Discriminant analysis. All three discriminant functions were highly significant (Table 5A). The first function explained 85% of the variability (Table 5A) and clearly discriminated between Santo Domingo and Topocalma locations (Fig. 3). From the relative magnitude of the discriminant function coefficients, we can determine how the independent variables (loci) were used to discriminate among the groups (Table 5A). The three loci, X7-1360, X7-570, and X17-1155, showed the highest coefficients of the first discriminant function, indicating that they were the most efficient loci for differentiating the two locations. The second function explained ca. 10% of the variance, where loci X17-2025 and X17-1750 were the most discriminating variables. Finally, the third function explained less than 5% of the variance, and loci X12-290 and X17-1125 were found to be the most discriminating variables. The predictions of the model were robust when comparing locations separated by 60 km, as only nine individuals from Santo Domingo were displayed in the Topocalma group and only one individual from Topocalma was misclassified in the Santo Domingo group (Table 5B). In total, 69% of the individuals were correctly classified in their original stand (Table 5B).

DISCUSSION

This study documents the level and distribution of RAPD loci variability within and among natural stands of the red alga *M. laminarioides*. Our results revealed

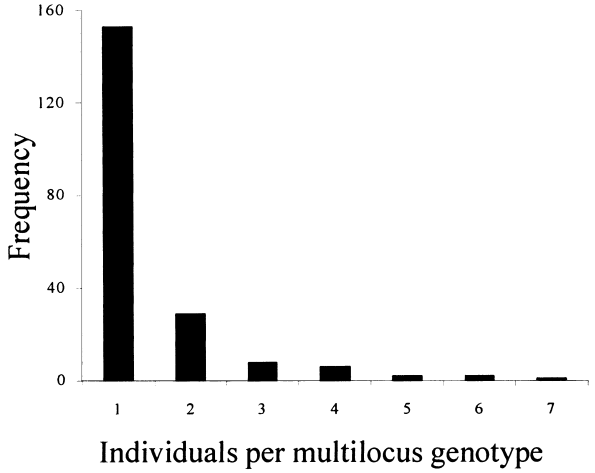

FIG. 2. Frequency distribution of unique (one individual) and shared (two to seven individuals) RAPD-multilocus genotypes within the whole set of analyzed *Mazzaella laminarioides* individuals ($n = 288$).

1) a high level of multilocus genotype, 2) a pattern of genetic structure that varied with the spatial scales, and 3) a strong genetic differentiation between locations that allowed the assignment of individuals to their original location through a canonical discriminant analysis.

Genetic diversity. In the present study, a high level of gene diversity was detected, as our results fit with the top range of *H* values reported for sexually reproducing red seaweed species (Pearson and Murray 1997, Sosa and Lindstrom 1999). However, direct comparison with available information on genetic diversity must be cautious, because most of the previously published data comes mainly from allozymes, which are generally less polymorphic than RAPD markers. Compared with land plants, the values of *H* observed within stands of marine algae in this study (from 0.21 to 0.25) are comparable or lower than those estimated with RAPD markers in obligate outcrossing species (*H* ranged from 0.22 to 0.29 for *Asimina triloba* [Huang et al. 2000] and from 0.34 to 0.48 for *Pinus cotorta* [Thomas et al. 1999]) but always higher than the values estimated in selfing or highly inbred land plant species (*H* ranged from 0.16 to 0.19 in the highly selfing *Hordeum spontaneum* [Owuor et al. 1997] and from 0.11 to 0.25 for inbred populations of the orchid *Goodyera procera* [Wong and Sun 1999]). Our results are thus consistent with the biological characteristics of the study species. Indeed, *M. laminarioides* is a dioecious, and thus obligatory outcross-

TABLE 4. Multilocus genotype diversity analysis.
(A) Number of distinct RAPD multilocus genotypes at each spatial scale.

	Sample size	Number of different RAPD genotypes (%)
Santo Domingo		
Quadrat A	16	13 (81.3%)
Quadrat B	16	16 (100%)
Quadrat C	16	12 (75.0%)
Quadrat D	16	15 (93.8%)
Total Santo Domingo	64	42 (65.6%)
Topocalma North		
Quadrat A	17	16 (94.1%)
Quadrat B	15	14 (93.3%)
Quadrat C	15	14 (93.3%)
Quadrat D	20	18 (90.0%)
Total Topocalma North	67	54 (80.6%)
Topocalma Middle		
Quadrat A	17	16 (94.1%)
Quadrat B	19	19 (100%)
Quadrat C	20	20 (100%)
Quadrat D	20	19 (95.0%)
Total Topocalma Middle	76	65 (85.5%)
Topocalma South		
Quadrat A	20	18 (90.0%)
Quadrat B	22	20 (90.9%)
Quadrat C	19	19 (100%)
Quadrat D	20	19 (95.0%)
Total Topocalma South	81	67 (82.7%)
Total Topocalma	224	162 (72.3%)
Overall samples	288	202 (70.1%)

(B) Number (and percentage) of shared multilocus genotypes among quadrats within each stand and between pairs of stands.

Stands	Santo Domingo	Topocalma North	Topocalma Middle	Topocalma South
Santo Domingo	11 (26.2%)			
Topocalma North	1 (1.1%)[a]	8 (14.8%)		
Topocalma Middle	2 (1.9%)[a]	5 (5.0%)	10 (15.4%)	
Topocalma South	1 (0.9%)[a]	12 (11.4%)	8 (6.5%)	10 (14.5%)

[a] Only two distinct multilocus genotypes were shared between Santo Domingo and Topocalma.

TABLE 5. Linear discriminant analysis within stands of *Mazzaella laminarioides* using the 17 RAPD locus as predictor variables and stands as the group classification variable.

(A) Percentage of variation explained by the three discriminant functions and the relative contribution of each variable (locus).

Variables	Correlation with the discriminant functions (standardized coefficients)			*P* level
	First function	Second function	Third function	(over the three functions)
X7-1360	0.817	−0.189	0.142	0.209
X7-1310	0.022	−0.253	−0.308	0.056
X7-1110	−0.118	0.209	−0.097	0.126
X7-570	−0.774	0.155	−0.144	$<10^{-6}$
X12-1250	0.171	0.201	0.059	0.067
X12-1100	0.203	−0.145	0.046	0.100
X12-820	0.074	−0.081	−0.181	0.589
X12-290	0.230	0.100	−0.596	$<10^{-4}$
X17-2025	−0.145	−0.552	−0.116	$<10^{-4}$
X17-1750	−0.370	0.378	0.197	$<10^{-6}$
X17-1400	−0.091	−0.403	0.236	0.002
X17-1155	0.584	0.106	−0.031	$<10^{-6}$
X17-1125	0.143	0.295	0.286	0.004
X17-700	−0.267	−0.401	0.256	$<10^{-5}$
A2-1025	−0.102	0.051	0.133	0.408
A2-900	−0.384	−0.179	−0.330	$<10^{-6}$
A2-740	−0.085	−0.022	0.212	0.388
Eigenvalues	3.217	0.400	0.163	
Relative percentage	85.12	10.58	4.30	
R Canonical correlation	0.873	0.534	0.374	
Wilk's lambda	0.146	0.615	0.860	
P level	$<10^{-6}$	$<10^{-6}$	$<10^{-3}$	

(B) Percentage of correctly classified individuals for each stand.

Predicted group	STDO	TOPO-North	TOPO-Middle	TOPO-South	Total observed	% Correctly classified
Observed group						
STDO	55	6	1	2	64	85, 94
TOPO-North	0	55	14	7	67	72, 37
TOPO-Middle	1	15	49	16	76	60, 49
TOPO-South	0	8	18	41	81	61, 19
Total predicted	56	84	82	66	288	69, 44

ing species in which apomixis has never been reported. Furthermore, fragments of *M. laminarioides* fronds tend to rapidly sink and decay, precluding the possibility of regeneration and reattachment to the substratum as has been observed for other red algae, like *Gelidium sesquipedale* (Juanes and Puente 1993) or *Lithotrix aspergillum* (Tyrell and Johansen 1995).

The number of different RAPD genotypes (202 for the 288 individuals, corresponding to 70% of distinct genotypes) observed within the overall samples is very high in comparison with other studies using multilocus DNA markers for detecting genotypic variation in clonal (or highly selfing) species. For example, only 81 RAPD phenotypes among 149 sampled individuals (54%) were reported in the red apomictic seaweed *Delisea pulchra*, in which up to 36 individuals shared the same multilocus genotype (Wright et al. 2000). In the clonal gorgonian coral *Plexaura kuna*, the percentage of distinct M13 DNA fingerprints averaged 50% over the different study reefs (Coffroth and Lasker 1998), whereas in the selfing plant species *Medicago truncatula*, only 62 distinct RAPD genotypes were observed over the 187 plants studied (33%, Bonnin et al. 1996). Furthermore, the percentage of distinct genotypes was quite similar from one stand to another in the current study (66% to 86%, Table 4), whereas in clonal and highly selfing species, this percentage is generally very variable from one population to another, ranging from 40% to 75% in *D. pulchra* (Wright et al. 2000), from 3% to 100% in *P. kuna* (Coffroth and Lasker 1998), or from 15% to 52% in *M. trunculata* (Bonnin et al. 1996). The genetic composition in clonal and highly selfing species is mainly determined by the number of migrants, whereas in an outcrossing species, in addition to the stochastic migration events, distinct genotypes are regularly produced by recombination.

Finally, additional support for repeated occurrence of recombination within *M. laminarioides* stands resides in the absence of significant linkage disequilibrium among RAPD loci. In a clonal or a highly selfing species, the lack of meiotic recombination among loci generates linkage disequilibria by fixing associations of alleles at different loci. In *M. trunculata*, the presence of significant linkage disequilibrium among RAPD loci was strongly associated to high levels of selfing (Bonnin et al. 1996). In conclusion, the pattern of genetic diversity shows clearly that *M. laminarioides* is essentially a sexually reproducing species.

Within-stand genetic structure (less than 30 m). In the present study, the AMOVA among quadrats (separated

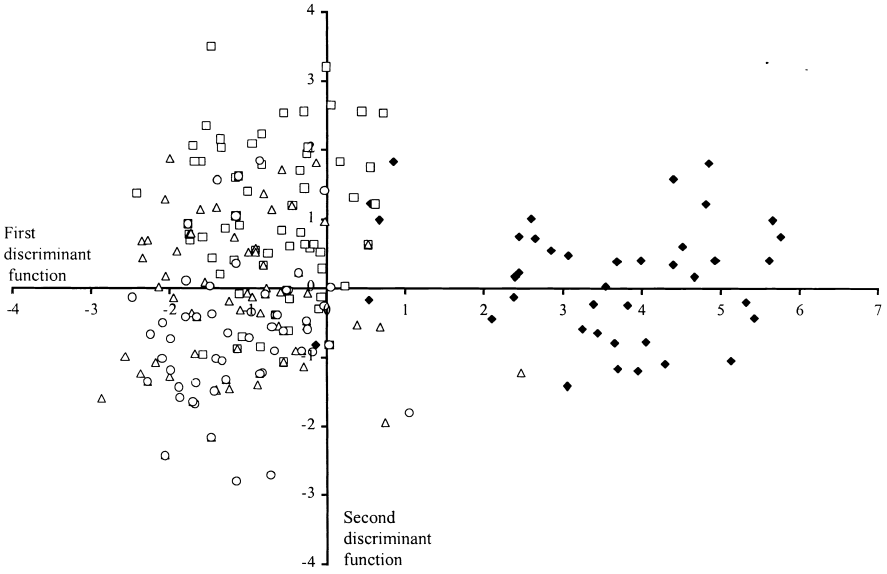

FIG. 3. Location of individuals on the two first discriminant functions (n = 288). Solid symbols refer to the Santo Domingo location and open symbols to the Topocalma location; within Topocalma, circles refer to Topocalma-North stand, squares to Topocalma-Middle stand, and triangles to Topocalma-South stand.

by 5 to 30 m within stands) revealed a weak and only marginally significant genetic structure. Such substructure was neither attributable to differentiation between quadrats within plots nor between plots within stands, indicating that genetic distances among individuals were not related to geographical distances at this spatial scale. In this context, it might be possible that our within-stand sampling did not include the pertinent geographical distances. For example, if dispersal is higher than 30 m, the covered area was too small to detect any kind of isolation by distance. This can be easily corrected by adding intermediate spatial scales ranging from 30 m to 5 km in the sampling design. Alternatively, factors other than strict geographical distances influence the genetic structure at the smallest scales. Even if dispersal abilities of spores and gametes actually reach at least 30 m, gamete encounters or spore settlement could be affected by a complex combination of local processes such as tidal water currents or heterogeneous habitat availability. It is in fact very difficult to get a good estimate of actual dispersal distances. For example, Ruckelshaus (1998) suggested that tidal currents could account for most of the discrepancies between genetic and geographical distances reported in many sessile marine organisms. In higher plants, the movements of pollinators or seed dispersing agents, rather than direct estimates of geographical distances, was shown to modulate the genetic structure of populations (Loveless and Hamrick 1984, Linhart and Grant 1996). Similarly, in *M. laminarioides*, some herbivores (i.e. amphipods) were reported to behave as seed dispersing agents in that they could transport spores in their digestive tract and facilitate their recruitment by providing a protected and nutrient-rich habitat (Buschmann and Vergara 1993). Consequently, the genetic structure of *M. laminarioides* stands is probably highly complicated by a combination of these different factors at the smallest spatial scales (few tens of meters).

An important issue regarding the observed pattern of within-stand genetic structure concerns the aggregated distribution of the pathogen *Pleurocapsa* sp. Our results indicate no relation between the presence of the pathogen and the distribution of genetic diversity. The lack of any clear pattern of differentiation among squares suggests that fertilization and recruitment do occur at distances of at least 30 m from the parental plants. Thus, the aggregated distribution of the pathogen does not appear to be explained by genetic substructuring among the host population.

Differentiation among stands (5 to 60 km). In this study, a significant genetic structure was systematically observed beyond the stand level (i.e. stands separated by at least 5 km). The clear trend of increasing genetic differentiation with increasing geographical distance indicates that genetic exchanges take place primarily between adjacent populations and not at random. Genetic differentiation among populations separated by 60 km was highly significant ($F_{ST} > 0.250$) and more than three times higher than that of stands separated by 5 km within the Topocalma location ($F_{ST} = 0.070$), whereas at lower scales (i.e. among quadrats within stands) only weak differentiation was observed ($F_{ST} = 0.025$). These results closely resemble those obtained with allozymes in different seaweed species characterized by limited dispersal, such as the red alga *Lithothrix aspergillum* (Pearson and Murray 1997) and the brown seaweed *Silvetia compressa* (as *Pelvetia fastigata*, Williams and DiFiori 1996). Even in the fucoid alga *Halidrys dioica*, characterized by drifting reproductive fragments responsible for the lack of small-scale genetic structure, a very strong differentiation occurred over distances of more than 70 km (Lu and Williams 1994). Studies based on molecular markers showed the same trend in the red algae *Gracilaria gracilis* (Engel et al. 1997) and in the kelp *Alaria marginata* (Kusumo and Druel 2000). Within the Topocalma location, the

strong genetic structure was mostly due to among-stands differentiation. Sandy beaches as short as 5 km are thus sufficient to generate substructuring, indicating that they constitute a barrier to gene flow. Restricted gene flow probably results from the limited long distance dispersal of spores, gametes, or drifting reproductive fragments. Even smaller gaps in the distribution of a species have been reported to behave as barriers to gene flow in other species such as land plants (Giles et al. 1998), marine gastropods (Johnson and Black 1998), and reef fishes (Riginos and Nachman 2001). These results suggest that gene flow occurs primarily among close or adjacent populations as described by the stepping stone model (Wright 1943): when stands are isolated by more than a few kilometers, gene flow is reduced and insufficient to counterbalance genetic drift.

Discriminant analysis. Population genetics studies estimate parameters derived from theoretical models. These models are based on evolutionary scenarios and ideal infinitely large populations. In this study, we have shown that these classical population genetic approaches provide valuable insights into our understanding of mating system and the effects of gene flow in *M. laminarioides*. In particular, indirect estimates of the magnitude of gene flow within and between populations have been interpreted in the context of spatial distribution of *M. laminarioides* stands. To complete this approach, the direction of gene flow can be detected by methods of assignment tests. More generally, Waser and Strobeck (1998) recently underlined the wide range of applications that the methods of assignment tests or related statistics might have in population biology (i.e. identifying individual dispersers, estimating rates of dispersal between populations, and locating the source habitat that sustains migratory individuals living in habitat sinks) and in several other fields such as forensics, conservation genetics, and stock management. This type of analysis explores the contribution of each variable (here, locus) to population structure, the number of which determines the statistical power. In this context, RAPD markers are particularly appropriate because they can produce a high number of non-linked loci.

In the present study, the discriminant analysis strongly differentiated populations of *M. laminarioides* separated by 60 km. The individuals could be assigned with high confidence to their original location by the first discriminant function. Only 10 of the 288 sampled individuals (3.5%) were not classified in their original location (Table 5B, Fig. 3). Of these misclassified individuals, only one was sampled in Topocalma and assigned to the Santo Domingo location (1/224, 0.5%), whereas the other nine were sampled in Santo Domingo but assigned to the Topocalma group (9/64, 14%). Moreover, the second discriminating function showed that within the Topocalma location, genetic differentiation among stands was significant. The method, however, was not efficient enough to discriminate among stands separated by 5 km, but we can expect that the assignment test could be improved by increasing the number of RAPD loci.

Although these results should be interpreted with caution because of the difference in sample sizes between the two locations, it is possible to consider the misclassified individuals as potential immigrants. In this context, the results suggest that the pattern of migration was not balanced between the two locations. Proportionally, more Santo Domingo individuals were misclassified than Topocalma individuals, indicating that Topocalma produced more migrants than Santo Domingo (because Topocalma genotypes were more frequently observed for individuals sampled within the Santo Domingo location than the reverse). This is in complete accordance with the descriptions of the movements of water masses, in particular the Humbolt Current, but also the principal coastal currents that are strictly south to north in central Chile (Hill et al. 1998). Thus, a spore or a gamete is more likely to disperse south to north, following the global movement of the water masses, that is from Topocalma to Santo Domingo.

In conclusion, here we illustrated by a variety of approaches how to take advantage of the enormous amount of information available in individual RAPD genotypes to detect gene movements among populations. Using the patterns of variation gleaned from only 17 RAPD loci, we have shown that *M. laminarioides* is a sexually reproducing species characterized by limited dispersal. Due to the discontinuous distribution of *M. laminarioides* stands along the Chilean coast, natural barriers (such as sandy beaches) may play a major role by increasing isolation among populations.

This study was supported by FONDECYT grant no. 1990145 to J.A.C and FONDECYT grant no. 1990235 to E.A.M, and Italian Ministry of Foreign Affairs, D.G.C.S., Italian-Chilean inter-university cooperation program; by the CNRS "Programme National Dynamique de la Biodiversité et Environnement" and "PICS: Programme Intégré de Coopération Scientifique"; and by ECOS-CONICYT cooperation program and the French embassy in Santiago (Chile) with a Lavoisier grant to S.F. We thank Carolyn Engel, two anonymous reviewers, and Susan Brawley for their useful comments on the manuscript.

Belkir, K., Borsa, P., Goudet, J., Chiki, L. & Bonhomme, F. 1996. *Genetix, logiciel sous WindowsTM pour la génétique des populations.* Laboratoire Génome et Populations, CNRS UPR 9060, Université Montpellier II, Montpellier, France.

Billot, C., Rousvoal, S., Estoup, A., Epplen, J. T., Saumitou-Laprade, P., Valero, M. & Kloareg, B. 1998. Isolation and characterisation of microsatellite markers in the nuclear genome of the brown alga *Laminaria digitata* (Phaeophyceae). *Mol. Ecol.* 7:1778–80.

Bohonak, J. A. 1999. Dispersal, gene flow, and population structure. *Q. Rev. Biol.* 74:21–45.

Bonnin, I., Huguet, T., Gherardi, M., Prosperi, J.-M. & Olivieri, I. 1996. High level of polymorphism and spatial structure in a selfing plant species, *Medicago trunculata* (Leguminosae), shown with RAPD markers. *Am. J. Bot.* 83:843–55.

Buschmann, A. H. & Vergara, P. A. 1993. Effect of rocky intertidal amphipods on algal recruitment: a field study. *J. Phycol.* 29:154–9

Coffroth, M. A. & Lasker, H. R. 1998. Population structure of clonal gorgonian coral: the interplay between clonal reproduction and disturbance. *Evolution* 52:379–93.

Correa, J. A., Flores, V. & Sánchez, P. 1993. Deformative disease in *Iridaea laminarioides* (Rhodophyta): gall development associated with an endophytic cyanobacterium. *J. Phycol.* 29:853–60.

Correa, J. A. & Sánchez, P. 1996. Ecological aspects of algal infectious diseases. *Hydrobiologia* 326/327:89–95.

Correa, J. A., Buschmann, A., Retamales, C. & Beltrán, J. 1997. Infectious diseases of *Mazzaella laminarioides* (Rhodophyta): changes in infection prevalence and disease expression associated with season, locality, and within-site location. *J. Phycol.* 33:344–52.

Coyer, J. A., Olsen, J. L. & Stam, W. T. 1997. Genetic variability and spatial separation in the sea palm kelp *Postelia palmaeformis* (Phaeophyceae) as assessed with M13 fingerprints and RAPDs. *J. Phycol.* 33:561–8.

Craigie, J. S. & Leigh, C. 1978. Carrageenans and agars. *In* Hellebust, J. A. & Craigie, J. S. [Eds.] *Handbook of Phycological Methods: Physiological and Biochemical Methods.* Cambridge University Press, London, UK, pp. 109–31.

Engel, C. R., Wattier, R., Destombe, C. & Valero, M. 1997. Dispersal in a haplo-diploïd red alga species: genetic structure and gene flow in *Gracilaria gracilis. Vie Milieu* 47:333–7.

Engel, C. R., Wattier, R., Destombe, C. & Valero, M. 1999. Performance of non-motile male gametes in the sea: analysis of paternity and fertilization success in a natural population of a red seaweed, *Gracilaria gracilis. Proc. R. Soc. Lond. B* 166:1879–86.

Excoffier, L., Smouse, P. E. & Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among restriction data. *Genetics* 131:479–91.

Faugeron, S., Martínez, E. A., Sánchez, P. A. & Correa, J. A. 2000. Infectious diseases in *Mazzaella laminarioides* (Rhodophyta): estimating the effect of infections on host reproductive potential. *Dis. Aquat. Org.* 42:143–8.

Giles, B. E., Lundqvist, E. & Goudet, J. 1998. Restricted gene flow and subpopulation differentiation in *Silene dioica. Heredity* 80:715–23.

Goudet, J. 1999. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9) Available at http: www.unil.ch/izea/softwares/fstat.html

Harris, S. A. 1999. RAPDs in systematics—a useful methodology? *In* Hollingsworth, P. M., Bateman, R. M. & Gornall, R. J. [Eds.] *Molecular Systematics and Plant Evolution.* Taylor & Francis, London, UK, pp. 211–28.

Hill, A. E., Hickey, B. M., Shillington, F. A., Strub, P. T., Brink, K. H., Barton, E. D. & Thomas, A. C. 1998. Eastern ocean boundaries: coastal segment. *In* Robinson, A. R. & Brink, K. H. [Eds.] *The Sea, Volume 11. The Global Coastal Ocean: Regional Studies and Synthesis.* J. Wiley & Sons, New York, NY, pp. 29–67.

Hoffmann, J. A. & Camus, P. 1989. Sinking rates and viability of spores from benthic algae in central Chile. *J. Exp. Mar. Biol. Ecol.* 126:281–91.

Huang, H., Layne, D. R. & Kubisiak, T. L. 2000. RAPD inheritance and diversity in Pawpaw (*Asimina triloba*). *J. Am. Soc. Hort. Sci.* 125:454–9.

Johnson, L. E. & Brawley, S. H. 1998. Dispersal and recruitment of a canopy-forming intertidal alga, *Pelvetia compressa* (Phaeophyceae). *Oecologia* 117:517–26.

Johnson, M. S. & Black, R. 1998. Increased genetic divergence and reduced genetic variation in populations of the snail *Bembicium vittatum* in isolated tidal ponds. *Heredity* 80:163–72.

Juanes, J. A. & Puente, A. 1993. Differential reattachment capacity of isomorphic life history phases of *Gelidium sesquipedale. Hydrobiologia* 260/261:139–44.

Knowlton, N. & Jackson, J. B. C. 1993. Inbreeding and outbreeding in marine invertebrates. *In* Thornhill, N. W. [Ed.] *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives.* University of Chicago, Chicago, pp. 200–49.

Kusumo, H. T. & Druel, L. D. 2000. Variability over space and time in the genetic structure of the winged kelp *Alaria marginata. Mar. Biol.* 136:397–409.

Lewontin, R. C. & Kojima, K. 1960. The evolutionary dynamics of complex polymorphisms. *Evolution* 14:450–72.

Linhart, Y. B. & Grant, M. C. 1996. Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Ecol. Syst.* 27:237–77.

Loveless, M. D. & Hamrick, J. L. 1984. Ecological determinants of genetic structure in plant populations. *Annu. Rev. Ecol. Syst.* 15:65–95.

Lu, T. T. & Williams, S. L. 1994. Genetic diversity and genetic structure in the brown alga *Halidrys dioica* (Fucales: Cystoseiraceae) in Southern California. *Mar. Biol.* 121:363–71.

Luo, H., Mörchen, M., Engel, C., Destombe, C., Epplen, J. T., Epplen, C., Saumitou-Laprade, P. & Valero, M. 1999. Characterisation of microsatellite markers in the red alga *Gracilaria gracilis. Mol. Ecol.* 8:700–2.

McFadden, C. S. & Aydin, K. Y. 1996. Spatial autocorrelation analysis of small-scale genetic structure in a clonal soft coral with limited larval dispersal. *Mar. Biol.* 126:215–24.

Meneses, I. 2001. Insights on the biology of *Gracilaria chilensis* using molecular techniques. *Cah. Biol. Mar.* 42:45–51.

Miller, K. A., Olsen, J. L. & Stam, W. T. 2000. Genetic divergence correlates with morphological and ecological subdivision in the deep-water elk kelp, *Pelagophycus porra* (Phaeophyceae). *J. Phycol.* 36:862–70.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–90.

Owuor, E. D., Fahima, T., Beiles, A., Korol, A. & Nevo, E. 1997. Population genetic response to microsite ecological stress in wild barley, *Hordeum spontaneum. Mol. Ecol.* 6:1177–87.

Pearson, E. A. & Murray, S. N. 1997. Patterns of reproduction, genetic diversity, and genetic differentiation in California populations of the geniculate coralline alga *Lithothrix aspergillum* (Rhodophyta). *J. Phycol.* 33:753–63.

Procaccini, G. & Mazzella, L. 1998. Population genetic structure and gene flow in the seagrass *Posidonia oceanica* assessed using microsatellite analysis. *Mar. Ecol. Prog. Ser.* 169:133–41.

Rice, W. R. 1989. Analysing tables of statistical tests. *Evolution* 43:223–5.

Riginos, C. & Nachman, M. W. 2001. Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus. Mol. Ecol.* 10:1439–53.

Ruckelshaus, M. H. 1998. Spatial scale of genetic structure and an indirect estimate of gene flow in eelgrass, *Zostera marina. Evolution* 52:330–43.

Santelices, B. 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanogr. Mar. Biol. Annu. Rev.* 28:177–276.

Santelices, B. 1991. Littoral and sublittoral communities of continental Chile. *In* Mathieson, A. C. & Nienhuis, P. H. [Eds.] *Ecosystems of the World.* Elsevier Science, New York, pp. 347–69.

Santelices, B., Correa, J. A., Meneses, I., Aedo, D. & Varela, D. 1996. Sporeling coalescence and intraclonal variation in *Gracilaria chilensis* (Gracilariales, Rhodophyta). *J. Phycol.* 32:313–22.

Santelices, B., Correa, J. A., Aedo, D., Flores, V., Homarzabal, M. & Sánchez, P. 1999. Convergent biological processes in coalescing Rhodophyta. *J. Phycol.* 35:1127–49.

Saunders, G. W. 1993. Gel purification of red algal genomic DNA: an inexpensive and rapid method for the isolation of polymerase chain reaction-friendly DNA. *J. Phycol.* 29:251–4.

Schneider, S., Roessli, S. & Excoffier, L. 2000. *ARLEQUIN. A Software for Population Genetics Data Analysis.* Version 2.000. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Shulman, M. J. 1998. What can population genetics tell us about dispersal and biogeographic history of coral-reef fishes? *Aust. J. Ecol.* 23:216–25.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science* 236:787–92.

Sosa, P. A. & Lindstrom, S. C. 1999. Isozymes in macroalgae (seaweeds): genetic differentiation, genetic variability and systematic applications. *Eur. J. Phycol.* 34:427–42.

StatSoft, Inc. 1995. *STASTISTICA for Windows.* Version 5.0. StatSoft Inc, Tulsa, OK.

Sunnucks, P. 2000. Efficient genetic markers for population biology. *Trends Ecol. Evol.* 15:199–203.

Thomas, B. R., Macdonald, S. E., Hicks, M., Adams, D. L. & Hodgetts R. B. 1999. Effects of reforestation methods on genetic diversity of lodgepole pine: an assessment using microsat-

ellite and randomly amplified polymorphic DNA markers. *Theor. Appl. Genet.* 98:793–801.
Tyrell, B. & Johansen, H. W. 1995. Reproductive and regeneration strategies of *Lithothrix aspergillum* (Corallinales, Rhodophyta) in Southern California. *Phycologia* 34:39–44.
Valero, M., Engel, C., Billot, C., Kloareg, B. & Destombe, C. 2001. Concepts and issues of population genetics in seaweeds. *Cah. Biol. Mar.* 42:53–62.
Waser, P. & Strobeck, C. 1998. Genetic signatures of interpopulation dispersal. *Trends Ecol. Evol.* 13:43–4.
Welsh, J. & McClelland, M. 1990. Fingerprinting genomes using PCR arbitrary primers. *Nucleic Acids Res.* 18:7213–8.
Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531–5.
Williams, S. L. & DiFiori, R. E. 1996. Genetic diversity and structure in *Pelvetia fastigiata* (Phaeophyta, Fucales): does a small effective neighborhood size explain fine-scale genetic structure? *Mar. Biol.* 126:371–82.
Wong, K. C. & Sun, M. 1999. Reproductive biology and conservation genetics of *Goodyera procera* (Orchidaceae). *Am. J. Bot.* 86:1406–13.
Wright, J. T., Zuccarello, G. C. & Steinberg, P. D. 2000. Genetic structure of the subtidal red alga *Delisea pulchra*. *Mar. Biol.* 136:439–48.
Wright, S. 1943. Isolation by distance. *Genetics* 28:114–38.
Yund, P. O. & O'Neil, P. G. 2000. Microgeographic genetic differentiation in a colonial ascidian (*Botryllus schlosseri*) population. *Mar. Biol.* 137:583–8.
Zuccarello, G. C., Yeats, P. H. & Wright, J. T. 2001. Population structure and physiological differentiation of haplotypes of *Caloglossa leprieurii* (Rhodophyta) in a mangrove intertidal zone. *J. Phycol.* 37:235–44.