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Assessing patterns of geographic dispersal of *Gelidium sesquipedale* (Rhodophyta) through RAPD differentiation of populations

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ABSTRACT: Randomly amplified polymorphic DNAs (RAPDs) of bulked genomic DNA samples were used to analyse the genetic differentiation of *Gelidium sesquipedale* populations. They reflect the patterns of gene flow, which in turn depend on the dispersal mechanisms of the species and on near-shore ocean currents. Fourteen populations were sampled from northern France to Morocco, covering the geographical distribution of the species. A single bulk DNA sample (from 15 individuals) was used in each population, under the assumption that the resulting patterns represent the populations' most common genetic features. To test this, we investigated the genetic variability among 5 bulk samples within a single population. Genetic distances among bulks were very low (average = 0.065) and were significantly lower than those observed between geographically separated populations (average = 0.241). Neighbour-joining analysis of the distance matrix of populations separated a well-supported group including populations of northern Spain and of northern France, and a less-supported group containing populations of northern Portugal. Multidimensional scaling of the genetic distance matrix revealed 2 isolated populations, São Rafael in southern Portugal and Algeciras in southern Spain. These patterns of genetic differentiation are discussed under the available data on the near-shore ocean currents. Results suggest that the genetic differentiation of *G. sesquipedale* populations may be used as a biological tracer of prevailing flows and barriers of the near-shore currents. A positive correlation between geographical and genetic distances of *G. sesquipedale* populations along the species geographical distribution was found, suggesting that a continuous transport of detached fronds and their reattachment to new substrate must be an effective dispersal mechanism of the species, sustaining the gene flow among populations.

KEY WORDS: *Gelidium sesquipedale* · RAPD · Population genetic variability · Geographical and genetic distances · Seaweed dispersal · Northeast Atlantic circulation · Biotracers

INTRODUCTION

Gelidium sesquipedale populations can be found along exposed shores of the Northeast Atlantic from Cornwall, southwest of Great Britain, to Mauritania (Dixon & Irvine 1977). The species is the most economically important agarophyte algae in the Northeast Atlantic, particularly in Spain, Portugal and Morocco (Juanes & Borja 1991, Santos & Duarte 1991, Melo 1998). It is the main source of raw material for specialised, highly priced products such as bacteriological grade agar and agarose.

Here we assessed the patterns of genetic differentiation of populations of *Gelidium sesquipedale* along the species geographical distribution. They reflect the patterns of gene flow, which in turn depend on the dispersal mechanisms of the species and on near-shore ocean currents. As the most important mechanism of seaweed dispersal along the shores seems to be the transport of broken thalli by currents (see Santelices 1990 for a review), there must be a significant relationship between the genetic distances among populations and their geographical distances. The patterns of genetic differentiation of seaweed populations may be used as biotracers of prevailing flows and barriers of the poorly defined near-shore ocean currents. To our knowledge,

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seaweeds have never been used as biological tracers unlike oceanic planktonic species (Bucklin et al. 1989, Stoehr et al. 1997), fishes and invertebrates (Saavedra et al. 1995, Borsa et al. 1997).

To analyse the genetic differentiation of *Gelidium sesquipedale* populations we used randomly amplified polymorphic DNAs (RAPDs), a PCR technique developed by Welsh & McClelland (1990) and Williams et al. (1990). It consists of the amplification of genomic DNA using short random primers and low annealing temperatures. The amplification products are separated by gel electrophoresis, and differences between genotypes are reflected as differences in the banding pattern. For a general review of this technique, see Weising et al. (1995). In seaweed studies, RAPDs have been used for species identification (Patwary et al. 1993, Dutcher & Kapraun 1994, Patwary & van der Meer 1994, Ho et al. 1995, Gonzalez et al. 1996, Meneses 1996), biogeographic studies (van Oppen et al. 1994, Pakker et al. 1996), and the determination of genetic relatedness among and within populations (Coyer et al. 1997). Van Oppen et al. (1996) assessed the limits of RAPDs for seaweed biogeography. They suggested that RAPD data are accurate in identifying populations at large spatial scales (hundreds to thousands of kilometres apart), but may fail at shorter geographical scales.

Bulk DNA samples of *Gelidium sesquipedale* populations were used in this work. A bulk sample can be conceived of as a sample of the population DNA (Yu & Pauls 1993). These authors state that, although some genetic information is lost, RAPD patterns resulting from bulked-DNA samples represent a signature for the most common genetic features of the population. Michelmore et al. (1991) found that rare RAPD markers could not be detected in bulks when the DNA sample that they were derived from represented less than 10% of the total DNA. Bulk methodology proved to be adequate for assessing the genetic similarities between 3 *G. sesquipedale* populations from southern Portugal (Alberto et al. 1997). To assess if a single bulk sample represented the most common genetic features of a population we investigated the genetic variability among bulk samples of the same population.

METHODS

DNA extraction and bulking followed the procedures of Alberto et al. (1997). Fourteen populations were sampled, covering the species geographical distribution (Fig. 1). Fifteen tufts were collected in each population, except for Roscoff where only 5 tufts were collected. To make sure that each sample corresponded to a different individual, only 1 erect frond was sampled from each tuft (Alberto et al. 1997). Bulk variability at the population level was investigated by analysing the genetic distances among 5 bulk samples of *Gelidium sesquipedale* collected along a 2.5 km stretch of Cape Espichel coast (Portugal).

Samples were examined under a stereo microscope to select 15 vigorous, epiphyte-free fronds for DNA extraction. The apical portions of branches were cut and washed in a series of tap water and detergent (Teepol), in distilled water and in double-distilled sterile water. Population bulks were obtained by mixing 200 mg (wet weight) of algal material from each individual in a large sterile mortar. The tissue was ground in liquid nitrogen using a pestle. All the powder within the mortar was collected into a 50 ml polypropylene centrifuge tube (Nalgene cat-3119), ensuring that each individual contributed an equal amount of tissue to the final mixture. In each tube, 18 ml of extraction buffer (200 mM Tris [pH 8.5], 250 mM NaCl, 25 mM EDTA, 0.5% SDS) was added. Samples were then gently mixed and incubated at 65°C for 30 min. This buffer-heating step resulted in clearer DNA samples than those obtained in Alberto et al. (1997). A volume of 9 ml of 3 M sodium acetate was added. Samples were gently agitated, and

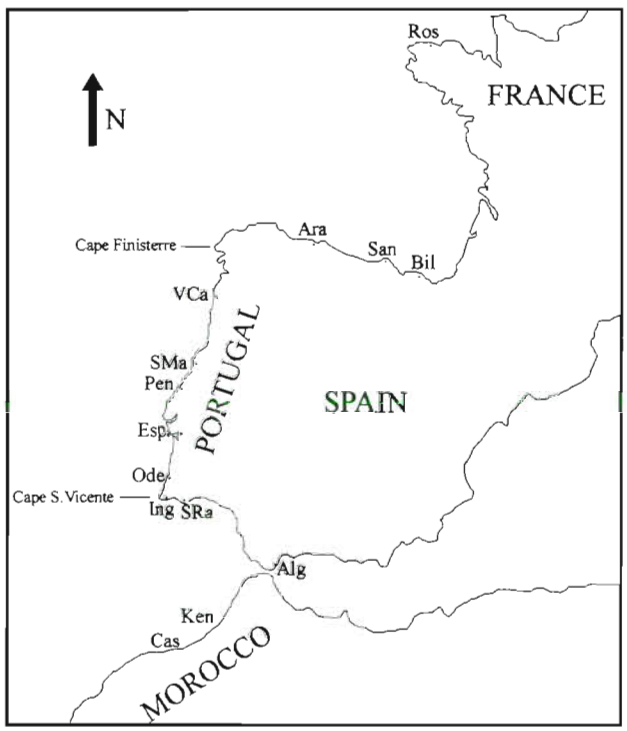

Fig. 1. Geographical location of *Gelidium sesquipedale* populations analysed in this study. Ros: Roscoff, Bil: Bilbao, San: Santander, Ara: Aramar, VCa: Viana do Castelo, SMa: São Martinho, Pen: Peniche, Esp: Espichel, Ode: Odeceixe, Ing: Ingrina, SRa: São Rafael, Alg: Algeciras, Ken: Kenitra, Cas: Casablanca

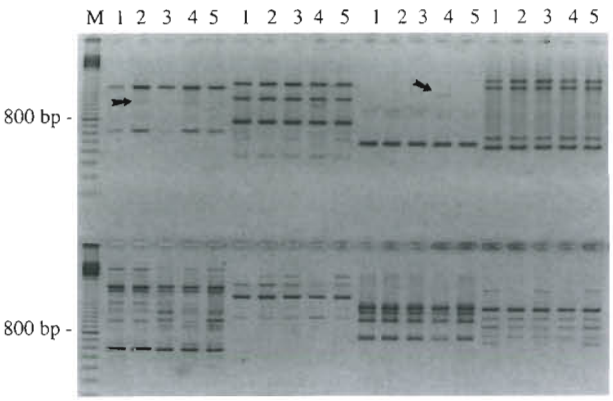

Fig. 2. RAPD amplifications of the 5 bulk samples of the *Gelidium sesquipedale* population of Espichel. Eight primers are presented: OPL13, OPL14, OPL15, OPL16, OPK4, OPK11, OPM5 and OPM6. Arrows indicate examples of 2 ambiguous bands. Lane M is the 100 base pair ladder molecular size marker, the larger band is 800 bp

then incubated at -20°C for 10 min. The mixture was centrifuged at 14 000 × *g* for 10 min. The supernatant was collected and precipitated with an equal volume of isopropanol (5 min at room temperature). The crude DNA extract was then centrifuged (14 000 × *g*, 5 min). The pellet was washed with ice cold 80% ethanol, centrifuged again (14 000 × *g*, 5 min), dried under vacuum and dissolved in 500 µl of Tris EDTA (TE) buffer. The quality of the bulk DNA samples was determined by agarose gel electrophoresis, and concentrations were estimated by comparisons with known quantities of prepurified calf thymus DNA (Pharmacia) (Sambrook et al. 1989).

Amplifications were performed in a total volume of 25 µl containing 2.5 µl 10× Taq DNA polymerase buffer (100 mM Tris HCl, pH = 9.0, 500 mM KCl, 15 mM $MgCl_2$), 150 µM of each dATP, dGTP, dCTP, dTTP, 0.4 µM of the primers, 20 ng genomic DNA and 1 unit Taq DNA polymerase (Pharmacia Biotech). PCR amplifications were programmed for a denaturation cycle of 1.5 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 36°C, 30 s at 72°C, and a final elongation cycle of 10 min at 72°C. Ramp times were 0.6°C s^{-1}. Amplification products were resolved by agarose gel electrophoresis (1.5%) in TBE buffer (45 mM Tris Borate, 1 mM EDTA) and visualised uner UV light after ethidium bromide staining.

One hundred 10-mer primers (Operon Technologies Inc., California, Kits A, K, L, M, N) were initially screened for amplification with *Gelidium sesquipedale* DNA. Those that did not show positive amplifications were immediately rejected. Each time a new population was analysed, primer reproducibility was tested by including a previously analysed population in the new run. Banding patterns of this population obtained at different occasions were compared. If the pattern was different the primer was considered non-reproducible, and therefore discarded.

A presence/absence data matrix of clearly defined bands produced by each primer was generated. Pairwise similarities (S) were calculated using both the simple matching coefficient (Sneath & Sokal 1973), number of all matches (including the number of absent bands in common) divided by the total number of bands, and the Dice coefficient (Dice 1945, Sneath & Sokal 1973), 2 times the bands shared by 2 samples, divided by the number of bands displayed by each sample. The first coefficient considers both the presence and the absence of bands to calculate similarity between samples while the second uses only the presence of bands. Pairwise similarities were converted into pairwise distances (1-S). Both coefficients revealed identical distance patterns. The Dice coefficient was chosen for all subsequent analyses. A multidimensional scaling analysis of the distance matrix was performed to reveal the relative genetic distance of populations in a space of 2 dimensions (Statistica package). A distance tree was constructed using the neighbour-joining method (Saitou & Nei 1987) of the program Distan 6.01 (Klerk & Stam unpubl.). Bootstrap analysis was done to test for tree reliability.

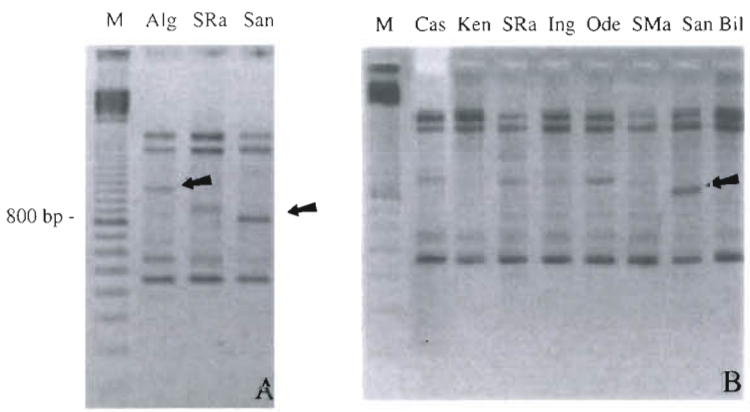

Fig. 3. RAPD amplifications of the following *Gelidium sesquipedale* populations, using primer OPL16: Alg: Algeciras, SRa: São Rafael, San: Santander, Cas: Casablanca, Ken: Kenitra, Ing: Ingrina, Ode: Odeceixe, SMa: São Martinho, Bil: Bilbao. The arrows show polymorphic markers specific for Algeciras and Santander. Polymorphism reproducibility is observed between gel A and B for the Santander lane

Table 1. Pairwise distance matrix among the 5 bulk samples collected in the *Gelidium sesquipedale* Espichel population. Distances were calculated as 1-Dice similarity

	Bulk 1	Bulk 2	Bulk 3	Bulk 4
Bulk 1				
Bulk 2	0.062			
Bulk 3	0.042	0.061		
Bulk 4	0.094	0.086	0.083	
Bulk 5	0.060	0.079	0.033	0.056

The correlation between genetic distance and geographic distance matrices was tested using the Mantel test (Mantel 1967) of the Genetix package (Belkhir et al. 1996–1998). This non-parametric test allows rejecting or retaining the null hypothesis that matrices are independent.

RESULTS

To assess the intrapopulation bulk variability, 18 primers were selected that generated 134 scorable bands. Seventeen primers were selected for interpopulation analysis, which generated 200 scorable bands. Only 22.3% of the bands of the intrapopulation analysis were polymorphic in contrast to the interpopulation analysis, where 71.5% of the bands were polymorphic. Fig. 2 illustrates RAPD amplifications of the 5 bulk samples from Espichel, using 8 primers. No variability was found among unambiguous bands when using these primers. Interpopulation amplifications using primer OPL16 are shown in Fig. 3. An example of reproducibility of a polymorphic band is evident between San lanes (Santander) of Fig. 3A,B.

Table 3. List of RAPD specific markers of *Gelidium sesquipedale* populations. Each marker was screened to reproducibility

Population	Primer	Base pair
São Rafael	OPL1	500
	OPL3	2100
	OPL3	2000
	OPL3	800
	OPL11	1800
	OPL13	900
Algeciras	OPL3	450
	OPL3	350
	OPL11	800
	OPL16	1100
Espichel	OPL3	1200
	OPL13	1800
Bilbao	OPA19	450
	OPL3	750
Santander	OPL8	580
	OPL16	800
Ingrina	OPA19	400
Peniche	OPL1	750
Viana do Castelo	OPL1	1800

Genetic distances among the 5 bulk samples collected at Espichel population (Table 1) were much lower than those among geographically separated populations (Table 2) The average distance among Espichel bulks was (0.065 ± 0.0196), significantly lower than the average distance among populations (0.241 ± 0.0426).

We were able to identify 18 markers that were specific for just 1 population (Table 3). An example of a population-specific marker is presented in Fig. 3A,B for Santander (Lane San). The number of specific polymorphic markers for São Rafael (6) and for Algeciras (4) was much higher than the other populations (1 to 2).

Table 2. Matrix of *Gelidium sesquipedale* geographical (km) (above diagonal) and genetic distances (D = 1-Dice similarity) (below diagonal) among populations. Populations are ordered from south to north: Cas: Casablanca, Ken: Kenitra, Alg: Algeciras, SRa: São Rafael, Ing: Ingrina, Ode: Odeceixe, Esp: Espichel, Pen: Peniche, SMa: São Martinho, VCa: Viana do Castelo, San: Santander, Ara: Aramar, Bil: Bilbao, Ros: Roscoff

	Cas	Ken	Alg	SRa	Ing	Ode	Esp	Pen	SMa	VCa	San	Ara	Bil	Ros
Cas	–	125	375	557	612	672	788	899	926	1170	1623	1805	1876	2536
Ken	0.161	–	250	532	587	647	763	874	900	1145	1598	1780	1851	2511
Alg	0.209	0.243	–	300	355	415	541	642	669	913	1366	1548	1619	2279
SRa	0.265	0.290	0.285	–	55	115	231	342	369	613	1066	1248	1319	1979
Ing	0.209	0.224	0.253	0.200	–	60	175	286	313	557	1010	1192	1263	1923
Ode	0.184	0.144	0.234	0.259	0.149	–	115	227	253	497	950	1132	1204	1864
Esp	0.246	0.238	0.278	0.237	0.237	0.167	–	111	138	382	835	1017	1088	1748
Pen	0.246	0.249	0.245	0.196	0.192	0.177	0.167	–	27	271	723	905	977	1637
SMa	0.203	0.206	0.270	0.215	0.183	0.154	0.231	0.133	–	244	697	879	950	1610
VCa	0.221	0.223	0.262	0.202	0.177	0.173	0.204	0.123	0.129	–	453	635	706	1366
San	0.212	0.193	0.311	0.290	0.236	0.272	0.295	0.249	0.241	0.268	–	182	253	913
Ara	0.209	0.179	0.282	0.296	0.289	0.223	0.301	0.245	0.213	0.24	0.158	–	71	731
Bil	0.178	0.169	0.263	0.299	0.223	0.203	0.259	0.236	0.193	0.243	0.148	0.115	–	660
Ros	0.213	0.183	0.303	0.340	0.252	0.207	0.275	0.252	0.209	0.259	0.161	0.117	0.116	–

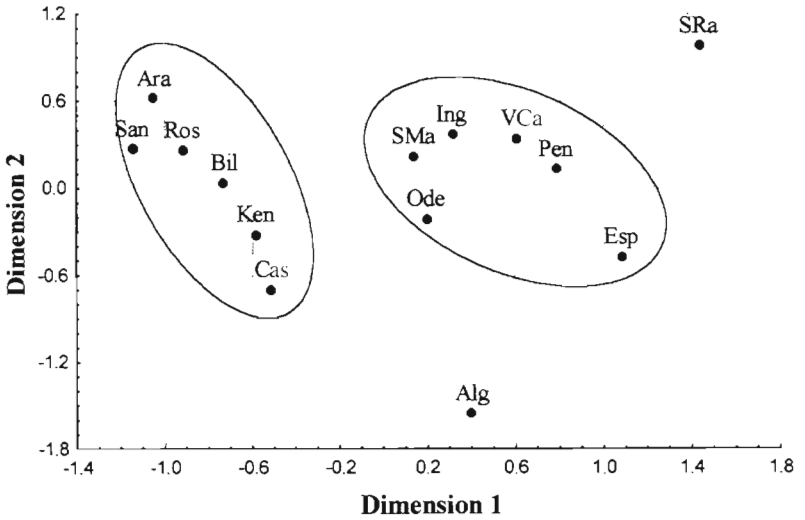

Fig. 4. Two-dimensional representation of multidimensional scaling analysis of the genetic distance matrix of *Gelidium sesquipedale*. Two groups of populations are evident (see text). Algeciras and São Rafael appear isolated. See Fig. 1 for population location abbreviations

This may be an indication of a low gene flow between these populations and their neighbours. Their relative isolation is also apparent when the distance matrix is analysed by multidimensional scaling (Fig. 4). This analysis made evident 2 main groups of populations: one including all the Portuguese populations except São Rafael, and the other including the populations of northern Spain, of France, and of Morocco.

Neighbour-joining analysis of the interpopulation distance matrix (Fig. 5) discriminated a northern group that includes the *Gelidium sesquipedale* populations from northern Spain, Aramar, Santander and Bilbao, and the population of Roscoff in France. This branch is supported by a high bootstrap value (98%), suggesting a clear genetic separation. Another group, which lay south from the above and contained populations from northern Portugal, Viana do Castelo, São Martinho and Peniche, also emerged, but was not as well supported by bootstrap analysis (85%).

RAPD genetic distances among *Gelidium sesquipedale* populations over the species geographic distribution showed a significant (Mantel test, $p = 0.024$) positive correlation with geographic distances among populations (Fig. 6).

DISCUSSION

Low genetic distances among bulks within the Espichel population, in relation to genetic distances of geographically separated populations, suggest that a single bulk may represent each population. Yu & Pauls (1993) showed that the RAPD patterns obtained from bulk DNA samples represent the most common markers of the individuals mixed in the bulk. Markers that are only present in a few individuals do not amplify, because they represent a small proportion of the template in the mixture, when compared to markers common to most individuals. This methodology only reveals well-conserved population markers, reducing faint bands and non-reproducible bands. This may be an advantage because faint bands contribute more to scoring error, hiding the RAPDs signal at short geographical scales (van Oppen et al. 1996).

Results showed that the lowest RAPD distances were found among northern populations, ranging from 0.115 to 0.161 (Table 2), suggesting higher gene flow within populations. This was evident not only among populations of the northern group, Aramar, Santander, Bilbao and Roscoff, but also among populations of northern Portugal, Viana do Castelo, São Martinho and Peniche (0.123 to 0.133). An explanation for this may be the fact that

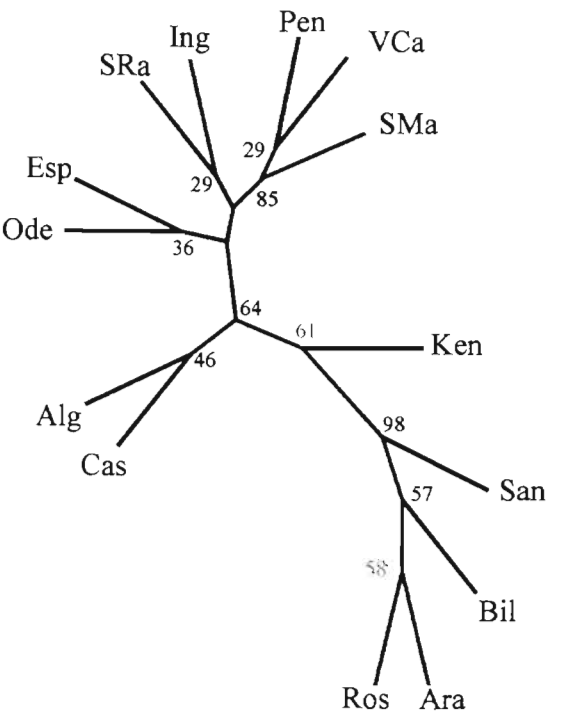

Fig. 5. Neighbor-joining distance tree of *Gelidium sesquipedale* populations. Numbers represent bootstrap values for each branch (100 replicates). See Fig. 1 for population location abbreviations

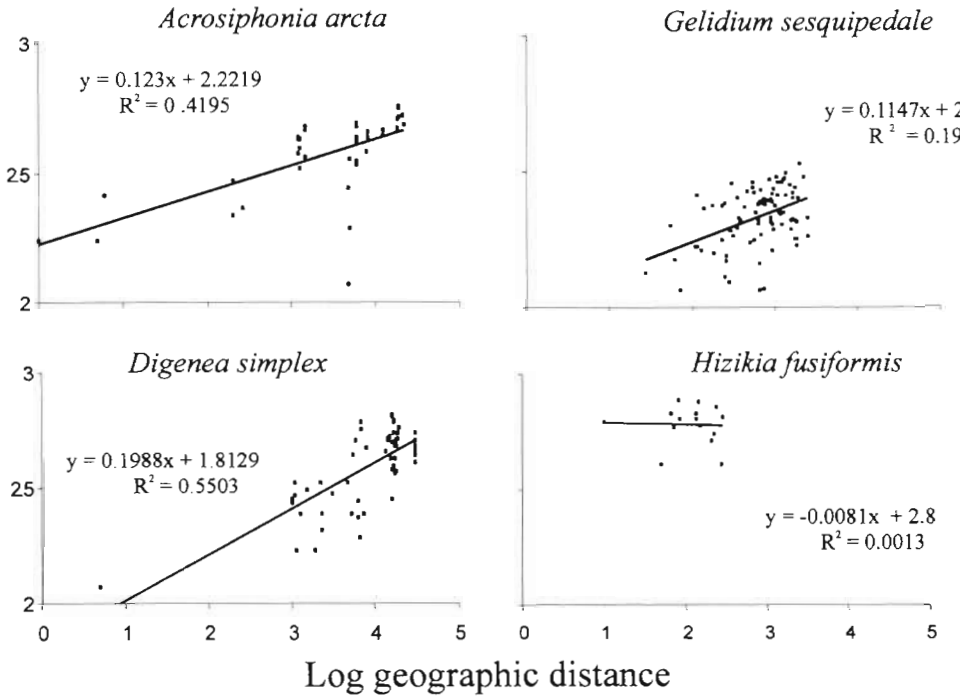

Fig. 6. Relationships between geographic and genetic distances among populations of 4 seaweed species, *Gelidiu sesquipedale* (this work), *Digenea simplex* (Pakker et al. 1996), *Acrosiphonia arcta* (van Oppen et al. 1994) and *Hizikia fusiformis* (Park et al. 1998). Mantel test was used to test for significant correlations between geographical and genetic distances (see text)

northern coasts are generally more stormy than southern ones, resulting in higher quantities of broken fronds in the water which may be transported to reattach in new locations. The dispersal strategies of *Gelidium* species rely more on the transport and reattachment of loose fronds than on spore dispersal. *Gelidium* spores do not disperse over long distances and have the strongest capacity for attachment immediately after shedding (Santelices 1990). On the other hand, the capacity of loose fronds of *G. sesquipedale* to re-attach to calcareous substrates, by production of rhizoidal clusters, has been well documented (Juanes & Puente 1993, Salinas & Valdés 1993). In addition, broken fronds can be fertile and shed their spores in new sites. Early work of Seoane-Camba (1969) in northern Spain showed both that the number of storm-tossed fronds of *G. sesquipedale* increased towards autumn, after stormy periods, and that more fertile fronds were found in tossed fronds than in the populations that remained attached.

Near-shore surface circulation along the Atlantic coastline of Europe is poorly defined, but there is evidence of both southward and northward flows. The upwelling events associated with the summer trade winds along the Portuguese coast result in a geostrophic adjustment that induces a southward circulation (Haynes et al. 1993). An example of seaweed transportation during summer was the presence of *Himanthalia elongata* receptacle blades at the southwest coast of Portugal (Santos pers. obs.). This locality lies a few hundred kilometres south of the southern limit of the species distribution, in northern Portugal (Ardré 1970). During the non-upwelling season, Frouin et al. (1990) and Haynes & Barton (1990) observed a surface poleward flow along the shelf edge of the western Iberian Peninsula. This suggests that there is a potential for high dispersal of fronds up and down the west coast of Portugal. Our results showed that genetic distances among Portuguese populations are generally lower than 0.2, except São Rafael (Table 2). In particular, the 85% bootstrap value for the grouping of Portuguese northern populations, Viana do Castelo, São Martinho and Peniche (Fig. 5), indicates high gene flow among them. This close association is not evident in the 2-dimensional plot of multidimensional scaling analysis of Fig. 4, but is evident along the third dimension (data not shown) which isolates the 3 populations.

Relvas & Barton (1996) observed a poleward flow along the continental shelf of the southern coast of Portugal around Cape São Vicente, in late spring, prior to upwelling. Upwelling events change the direction of this flow to toward the equator. The great mixing of water masses around Cape São Vicente may enhance the gene flow between Ingrina and Odeceixe. Ingrina, located along the southern coast is genetically more similar to Odeceixe, on the southwest coast than to São Rafael, located on the south coast near Ingrina (Fig. 1). Genetic distances among these populations showed the same similarity patterns when independently analysed elsewhere (Alberto et al. 1997). This is a good indication of the reproducibility of this methodology.

More evidence of the relative isolation of the São Rafael population is given by the multidimensional analysis (Fig. 4) and by its high number of polymorphic markers (Table 3). The same arguments suggest that Algeciras is also isolated. This population is situated in the northwest Alboran Sea, where water circulation anticyclone gyres are well documented (Viúdez et al. 1996). This causes a physical barrier that restricts the gene flow between Mediterranean and Atlantic surface water masses, as it was documented for fishes and invertebrates (Saavedra et al. 1995, Borsa et al. 1997).

The neighbour-joining analysis among *Gelidium sesquipedale* populations separated the most northern populations (from northern Spain and northern France) from all the others. This isolation suggests that Cape Finisterra is an important geographical barrier between the west coast of Portugal and the northern coast of Spain, preventing a regular transport of *G. sesquipedale* fronds around the northwest corner of the Iberian Peninsula (Cape Finisterra). There is controversy concerning the near-shore surface currents in this area. Some authors postulate that the Iberian northward current connects to the Porcupine current, along the west Scottish coast (Haynes & Barton 1990), whereas others show evidence that at least part of the current turns around Cape Finisterra (Haynes et al. 1993). However, the latter conclusion was based on the tracking of only 2 drifters from which only 1 turned around the cape. Model simulations developed by Neves et al. (1996) support this last hypothesis, contrary to our data which suggest that the long-term prevailing currents do not flow around Cape Finisterra. The use of seaweed genetic distances as biotracers of prevailing currents is advantageous because it integrates long-term patterns of water movement while many oceanographic observations are limited to single moorings or a few weeks of observations (Barton 1990).

The position of Moroccan populations in the multidimensional scaling plot (Fig. 4), lying closer to northern Spanish populations than to Portuguese populations, seems contradictory with both the geographical distances and the expected surface poleward flow along the coasts of northwest Africa and the western Iberian Peninsula (Barton 1990). It suggests that the transport of *Gelidium sesquipedale* from the Moroccan coast to the coast of Portugal is not frequent perhaps due to the long distance between them. The physical discontinuity where the Mediterranean outflow occurs further complicates the circulation between the northern Moroccan coast and the coast of Portugal.

To our knowledge, the correlation between genetic and geographical distances has not been tested in seaweed biogeographic studies. Fig. 6 compares the relationship between RAPD calculated genetic distance and geographical distance for *Gelidium sesquipedale*, with available data from *Acrosiphonia arcta* (van Oppen et al. 1994), *Digenea simplex* (Pakker et al. 1996) and *Hizikia fusiformis* (Park et al. 1998). A significant correlation was found for the first 3 species (Mantel test p-values, respectively, $p = 0.024$, $p = 0.00799$ and $p = 0.00499$), but not for *H. fusiformis* ($p = 0.658$). The decreasing p-values (increasing significance of correlation) follow the increase of geographical scales assessed, *H. fusiformis* being the lowest geographical scale study. The use of RAPDs to assess the genetic relatedness among seaweed populations is probably restricted to geographical scales higher than this, as van Oppen at al. (1996) have pointed out.

Both *Digenea simplex* and *Acrosiphonia arcta* are ancestral species with disjunct populations distributed worldwide. The low genetic relatedness among their isolated groups of populations results from long geological time scale processes such as paleoclimatic change and continental drift, rather than 'short time' dispersal mechanisms. On the other hand, the significant positive correlation showed in the case of *Gelidium sesquipedale* is evidence that a continuous transport of detached fronds by prevailing currents and their reattachment to new substrate is an effective dispersal mechanism of the species, sustaining the gene flow among populations.

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