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Genetic variability and differentiation of sporophytes and gametophytes in populations of *Gelidium arbuscula* (Gelidiaceae: Rhodophyta) determined by isozyme electrophoresis

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Abstract. Genetic differentiation and genetic variability of sporophytic and gametophytic populations of *Gelidium arbuscula* (Bory) from three localities sampled in 1989 and 1990 in the Canary Islands (Spain) were examined by isozyme electrophoresis. Twenty-three to 29 putative alleles corresponding to 22 gene loci, were compared. High deviations in Hardy-Weinberg equilibrium, and significant differences between allelic frequencies of sporophytic and gametophytic subpopulations at the same locality were found, suggesting a predominant asexual reproduction of *G. arbuscula*. The genetic variability (percentage of polymorphic loci, mean number of alleles per locus and average gene diversity) of haploid subpopulations was lower than that of diploid subpopulations at all three localities, being the lowest described for seaweeds. No correlation between genetic and geographical distance was found. The high genetic differentiation coefficient between all subpopulations suggests a very reduced genetic flow between subpopulations of the same and of different localities. These results suggest that the genetic structure of the populations of *G. arbuscula* from the Canary Islands is due to a founder-effect combined with a predominance of asexual reproduction. This is the first report comparing allelic frequencies between sporophytic and gametophytic subpopulations of seaweeds.

Introduction

Very little is known about genetic variability and differentiation among populations of marine algae (Innes 1984, Cheney 1985). Quantitative estimates of genetic variability and differentiation have been greatly facilitated by isozyme electrophoresis (Innes 1984). Studies of isozyme variability in seaweeds have been extensive for systematic purposes (Mallery and Richardson 1971, 1972, Miura et al. 1978a, Marsden et al. 1981, 1984a, b, Blair et al. 1982, Vilter and Glombitza 1983, Rice and Crowden 1987, Lindstrom and South 1989, Lindstrom and Cole 1990), whereas the application of isozyme electrophoresis to analyze genetic differentiation in populations of seaweeds has been very limited compared with other organisms (Innes 1984). Malinowsky (1974) analyzed ten populations of *Codium fragile* and found no differentiation between them. Innes (1987, 1988) described patterns of differentiation among populations of *Enteromorpha linza*, and suggested its maintenance by factors operating on a microgeographic scale. Data on red seaweed are very scarce. Cheney and Mathieson (1979) analyzed eight populations of *Chondrus crispus*, and also suggested the existence of considerable genetic differentiation over relatively short distances. Cheney and Babbel (1978) analyzed four species of *Eucheuma* and found a high genetic identity within *E. isiforme* and *E. nudum*. High genetic differentiation by isozyme electrophoresis was found between Japanese populations of *Porphyra yezoensis* (Miura et al. 1979, Fujio et al. 1985, 1987), but no data are available on agarophytes, the most commercially valuable of the seaweeds.

Fujio et al. (1985, 1987) reported the genetic differentiation between gametophytic populations of *Porphyra yezoensis* to be different from that of diploid organisms, but the amount of genetic variability did not differ much from that of diploid organisms including plants. No study has so far analysed and compared sporophytic and gametophytic subpopulations of seaweeds.

Materials and methods

Sampling

Three populations of *Gelidium arbuscula* (Bory) from three locations in the Canary Islands were examined. Sampling sites are shown in Fig. 1. Plants from a population were collected over a 15 to 20 m^2 area throughout the intertidal zone, at least twice a year during 1989 and 1990. In order to avoid sampling the same individual, single collections were made from aggregations which were at least 20 cm apart, usually on separate rocks. Each individual was transported to the laboratory in a separate plastic bag in an ice cooler. In the laboratory the samples were carefully cleaned of epiphytes and sorted into two subpopulations: tetrasporophytic

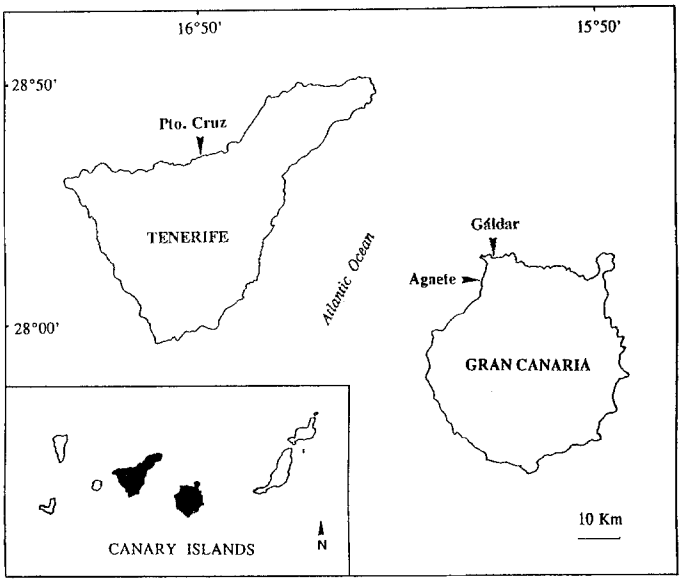

Fig. 1. Map of three sampling localities (arrowed) of *Gelidium arbuscula* in the Canary Islands

(bearing tetrasporangia) and gametophytic (bearing cistocarps). Prior to extraction of enzymes, plants were cultured in 10-litre tanks, under uniform culture conditions: 18 plants per 10 litres of Provasoli-enriched seawater (Provasoli 1968); 21 °C; 18 $\mu E\ m^{-2}\ s^{-1}$ (Gro-Lux, 1:1 cool-white fluorescent lamps); 16 h light:8 h dark; for periods ranging from 1 to 20 d. After 2 mo, the plants were still healthy, and showed no alteration in their enzyme-banding patterns.

Explants (0.30 to 0.5 g) were ground with mortar and pestle in liquid nitrogen until the sample was reduced to a fine powder, before the addition (1:1 w/v) of the grinding buffer. Preliminary experiments to determine the most effective procedure for grinding revealed the best grinding buffer assay to be 0.1 *M* Tris-HCl, pH 7.4, 0.2 *M* L-ascorbic acid, 1 m*M* Na_2 EDTA, 3% (w/v) polyvinyl-polypyrrolidone (PVPP).

The tissue-buffer mix was allowed to thaw and the crude extracts were absorbed onto paper wicks (Whatman N°.3; 1 × 0.3 cm) which were inserted vertically into the starch gel (7.5 × 22.5 × 4.5 cm).

Electrophoresis

Proteins were separated by horizontal starch-gel electrophoresis. Starch gels were prepared using 12% (w/v) Sigma starch in 0.015 *M* Tris, 0.003 *M* citric acid, pH 7.8, gel buffer. The electrode buffer consisted of 0.304 *M* boric acid, 0.1 *M* NaOH, pH 8.6 (Poulik buffer system, Cheney 1985). Tris-EDTA-borate, pH 8.6, and Tris-citrate, pH 7.2, buffer systems were also tested, but did not prove as efficient.

The gels were run at 250 V for 10 min, after which the wicks (18 to 20 per gel) were removed. The run was then continued in the same electrical conditions until the tracking dye (bromophenol blue) was 10 cm from the origin (after 3 to 3.5 h).

Following electrophoresis, the gels were cut horizontally into 3 or 4 slices and assayed (anodical and cathodical migration) for the following enzymes (to 100 ml final volume): alkaline phosphatase (ALP; E.C. 3.1.3.1) 0.1 g Fast Blue RR salt, 1.0 g NaCl, 0.1 g $MgCl_2$, 0.05 g $MnCl_2$, in 0.1 *M* Tris-HCl, pH 8.0 (modified from Ayala et al. 1972); NADH-diaphorase (DIA; E.C. 1.6.4.3) 12 mg NADH, 3 ml dimethylthiazoldiphenyl-tetrazolium (MTT) 1% (w/v), 5 mg dichlorophenol indophenol (DCPIP) in 0.1 *M* Tris-HCl, pH 8.0 (modified from Vallejos 1983); esterase (EST; E.C. 3.1.1.1) 0.1 g Fast Blue RR salt, 10 ml β-naphthyl acetate (1% (w/v) in acetone), in 0.1 *M* Tris-HCl, pH 7.1 (modified from Shaw and Prasad 1970); glutamate dehydrogenase (GDH; E.C. 1.4.1.2) 25 ml 1.5 *M* L-glutamic acid, pH 8.0, 40 mg NAD, 3 ml MTT 1% (w/v), 0.5 ml phenazine methosulphate (PMS) 1% (w/v) in 0.1 *M* Tris-HCl, pH 8.0 (modified from Cheney 1985); malate dehydrogenase (MDH; E.C. 1.1.1.37), same conditions as for GDH, but with 1.5 *M* L-malic acid substituted for L-glutamic acid; phosphoglucose isomerase (PGI; E.C. 5.3.1.9) 0.1 g fructose-6-phosphate, 10 mg NADP, 0.1 g $MgCl_2$, 3 ml MTT 1% (w/v), 0.5 ml PMS 1% (w/v), 40 U glucose-6-phosphate dehydrogenase, in 0.1 *M* Tris-HCl, pH 8.0 (modified from Soltis et al. 1983); phosphoglucose mutase (PGM; E.C. 2.7.5.1), same conditions as PGI, but with 0.1 g glucose-1-phosphate substituted for fructose-6-phosphate; superoxide dismutase (SOD; E.C. 1.15.1.1) 0.1 g Na_2 EDTA, 3 ml MTT 1% (w/v), 5 mg riboflavin, in 0.1 *M* Tris-HCl, pH 7.4 (20 min in the dark, and then between 1 to 2 h in the light until bands appear; modified from Vallejos 1983).

Other enzymatic systems were tested: alcohol dehydrogenase, aldolase, amylase, glutamate oxalacetate transaminase, catalase, malic enzyme (NADP-dependent), acid phosphatase, galactose dehydrogenase, α-glycero phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, hexokinase, isocitrate dehydrogenase, lactate dehydrogenase, leucine aminopeptidase, peroxidase, sorbitol dehydrogenase, shikimate dehydrogenase and xantine dehydrogenase; but, after several modifications, no activity was detected.

Individuals from different localities were run together, side by side on the same gel, to estimate whether corresponding electromorphs had similar mobilities. Stain solution without substrate served as controls. After staining, the gels were fixed in 50% ethanol and 3.5% acetic acid.

Banding-pattern interpretation

As formal genetic conclusions were not possible (sexual crosses were not performed) genetic interpretations of non-artefactual banding-patterns were inferred directly from isozyme phenotypes. A tentative interpretation of banding patterns was possible through identification of haploid (gametophytic) and diploid (tetrasporophytic) plants (Cheney and Babbel 1978).

Allelic designations were based primarily upon whether a banding-pattern displayed polymorphic or monomorphic characteristics; i.e., bands in haploid individuals were assumed to be the products of different loci, since only one allele (of the same locus) can occur in haploid individuals. Likewise, bands in the same activity zone which occurred together in diploid plants (heterozygotic) were considered to be alleles of the same locus (Cheney and Babbel 1978). The subunit structure of enzymes was also considered in the interpretation of banding-patterns; heterozygotes for a locus coding a dimeric enzyme will produce three bands, while heterozygotes for a locus coding a monomeric enzyme will produce two bands (Innes 1984).

Phycoerythrin (biliprotein, BP) was visible as a fluorescent band under ultraviolet light, and was used as an internal standard for comparative studies of band mobility. Presumed loci were labelled with the abbreviation for the enzyme name and numbered beginning with the locus displaying the highest anodal migration. Alleles of the same locus were designated with lowercase letters, starting with "a" for the highest anodal electrophoretic migration. Relative mobility, R_f (mobility of the band/mobility of bromophenol blue) was calculed for each electromorph.

Data analyses

The proportion of polymorphic loci (P), mean number of alleles per locus (A/L) and average heterozygosity or average gene diversity (*H*) (Nei 1987), were calculated to determine the amount of genetic variability for each subpopulation.

For each locus in Hardy-Weinberg equilibrium, the expected genotypic frequencies were calculated assuming sexually reproducing populations (panmixia). Since *Gelidium arbuscula* displayed alternation of generations, the allelic frequencies of gametophytes were treated as allelic frequencies of gametes in the population. Under conditions of panmixia, the allelic frequencies of male and female gametophytic subpopulations are identical, and the expected genotypic frequencies of sporophytic subpopulations, assuming mating crosses between male and female gametophytes, must be identical to those theoretically predicted by the Hardy Weinberg law. Therefore, expected genotypes of sporophytic subpopulations were calculated from the allelic frequencies of female gametophytic subpopulations from the same locality. Levene's formula for small samples (Levene 1949) was used to estimate the expected number of sporophytic individuals in Hardy-Weinberg equilibrium.

Chi-square tests (Nei 1987) were used to determine differences in allelic frequencies between haploid and diploid subpopulations for each locus. Chi-square values derived from independent loci were added together following the method of Workman and Niswander (1970).

Genetic identity, *I*, and genetic distance, *D* (Nei 1972), were computed for pairwise comparisons of the three localities and for pairwise comparisons of subpopulations within each locality. A dendrogram of genetic distances between subpopulations was constructed by UPGMA (unweighted pair-group method of analysis) cluster-analysis (Nei 1987).

Genetic differentation (and therefore gene flow) between subpopulations was estimated using Nei's coefficient of differentiation (G_{ST}) (Nei 1973). G_{ST} is a measure of the amount of differentiation among subpopulations, and indicates the genetic differentiation between alleles of different populations. G_{ST} can vary from 0 (= populations with same allele frequencies at locus in question) to 1 (= populations fixed for different alleles).

Results

Table 1 lists electromorphs (alleles) and shows their relative mobility (R_f) for each loci in *Gelidium arbuscula*. Fig. 2 presents typical banding patterns for ALP, DIA, PE, GDH, MDH and PGM. All bands migrated anodally. *ALP-1**, *EST-2**, *GDH-1** and *DIA-2** conformed to a monomeric structure enzyme, with two bands occurring in heterozygotic sporophytes (Fig. 2), whereas *MDH-1**, *MDH-2** and *PGI-2** conformed to two allele, dimeric enzymes, with three bands in heterozygotic sporophytes. We identified 22 gene loci, 7 of which (*ALP-1**, *DIA-2**, *EST-2**, *GDH-1**, *MDH-1**, *MDH-2** and *PGI-3**) were polymorphic in at least one subpopulation (Table 1).

The allelic frequencies of polymorphic loci are listed in Table 2. *DIA-2** displayed the greatest amount of variability (all subpopulations exhibited polymorphism), while *ALP-1** and *PGI-3** each exhibited polymorphism in one subpopulation only (Agaete sporophyte and Puerto Cruz sporophyte, respectively). Three alleles were observed for *DIA-2** and *GDH-1**, and two alleles for the remaining loci.

Table 3 summarizes the genetic variability in *Gelidium arbuscula* subpopulations. The diploid subpopulation (tetrasporophytic) displayed higher variability than the haploid subpopulation for all parameters in each locality. The percentage of polymorphic loci ranged from 4.5 to 27.3%, the average for the combined sporophyte subpopulations was 21.2%, and for the combined gametophytic subpopulations 6%. The mean for all subpopulations combined was 13.6%. The average number of alleles per locus ranged from 1.04 to 1.32; a mean of 1.06 for haploid subpopulations, and 1.24 for diploid subpopulations, 1.15 being the average for the species. The average gene diversity for sporophytic subpopulations was 0.070, for gametophytic subpopulations 0.018; 0.044 was the average for the species.

Table 1. *Gelidium arbuscula*. Relative mobilities (R_f) of alleles detected in sporophytic and gametophytic subpopulations from Canary Islands

Invariable loci		Polymorphic loci	
Alleles	R_f	Alleles	R_f
*PE**	0.38	*ALP-1* a*	0.43
*DIA-1**	0.85	*ALP-1* b*	0.38
*DIA-3**	0.59	*DIA-2* a*	0.68
*DIA-4**	0.50	*DIA-2* b*	0.64
*EST-1**	0.82	*DIA-2* c*	0.62
*EST-3**	0.35	*EST-2* a*	0.60
*PGI-1**	0.62	*EST-2* b*	0.58
*PGI-2**	0.60	*GDH-1* a*	0.44
*PGM**	0.79	*GDH-1* b*	0.38
*SOD-1**	0.90	*GDH-1* c*	0.36
*SOD-2**	0.85	*MDH-1* a*	0.73
*SOD-3**	0.77	*MDH-1* b*	0.62
*SOD-4**	0.75	*MDH-2* a*	0.55
*SOD-5**	0.73	*MDH-2* b*	0.53
*SOD-6**	0.70	*PGI-3* a*	0.55
		PGI-3 b*	0.49

Table 2. *Gelidium arbuscula*. Allelic frequencies of polymorphic loci detected in sporophytic (S) and gametophytic (G) subpopulations from three locations in Canary Islands. (*n*): no. of individuals analyzed

Allele	Gáldar		Agaete		Puerto Cruz	
	S	G	S	G	S	G
ALP-1 a*	0.00	0.00	0.09	0.00	0.00	0.00
ALP-1 b*	1.00	1.00	0.91	1.00	1.00	1.00
	(42)	(12)	(49)	(16)	(38)	(13)
DIA-2 a*	0.00	0.00	0.03	0.00	0.70	0.80
DIA-2 b*	0.37	0.10	0.88	0.73	0.30	0.20
DIA-2 c*	0.63	0.90	0.09	0.27	0.00	0.00
	(31)	(20)	(48)	(22)	(46)	(15)
EST-2 a*	0.50	1.00	0.64	1.00	0.79	1.00
EST-2 b*	0.50	0.00	0.36	0.00	0.21	0.00
	(45)	(12)	(38)	(15)	(31)	(16)
GDH-1 a*	0.39	0.17	0.00	0.00	0.00	0.00
GDH-1 b*	0.52	0.83	0.87	1.00	1.00	1.00
GDH-1 c*	0.09	0.00	0.13	0.00	0.00	0.00
	(33)	(12)	(39)	(19)	(35)	(11)
MDH-1 a*	1.00	1.00	0.79	1.00	0.69	1.00
MDH-1 b*	0.00	0.00	0.21	0.00	0.31	0.00
	(46)	(15)	(48)	(14)	(31)	(13)
MDH-2 a*	0.00	0.00	0.02	0.00	0.19	0.00
MDH-2 b*	1.00	1.00	0.98	1.00	0.81	1.00
	(43)	(14)	(48)	(18)	(31)	(11)
PGI-3 a*	0.00	0.00	0.00	0.00	0.07	0.00
PGI-3 b*	1.00	1.00	1.00	1.00	0.93	1.00
	(39)	(12)	(49)	(19)	(37)	(14)

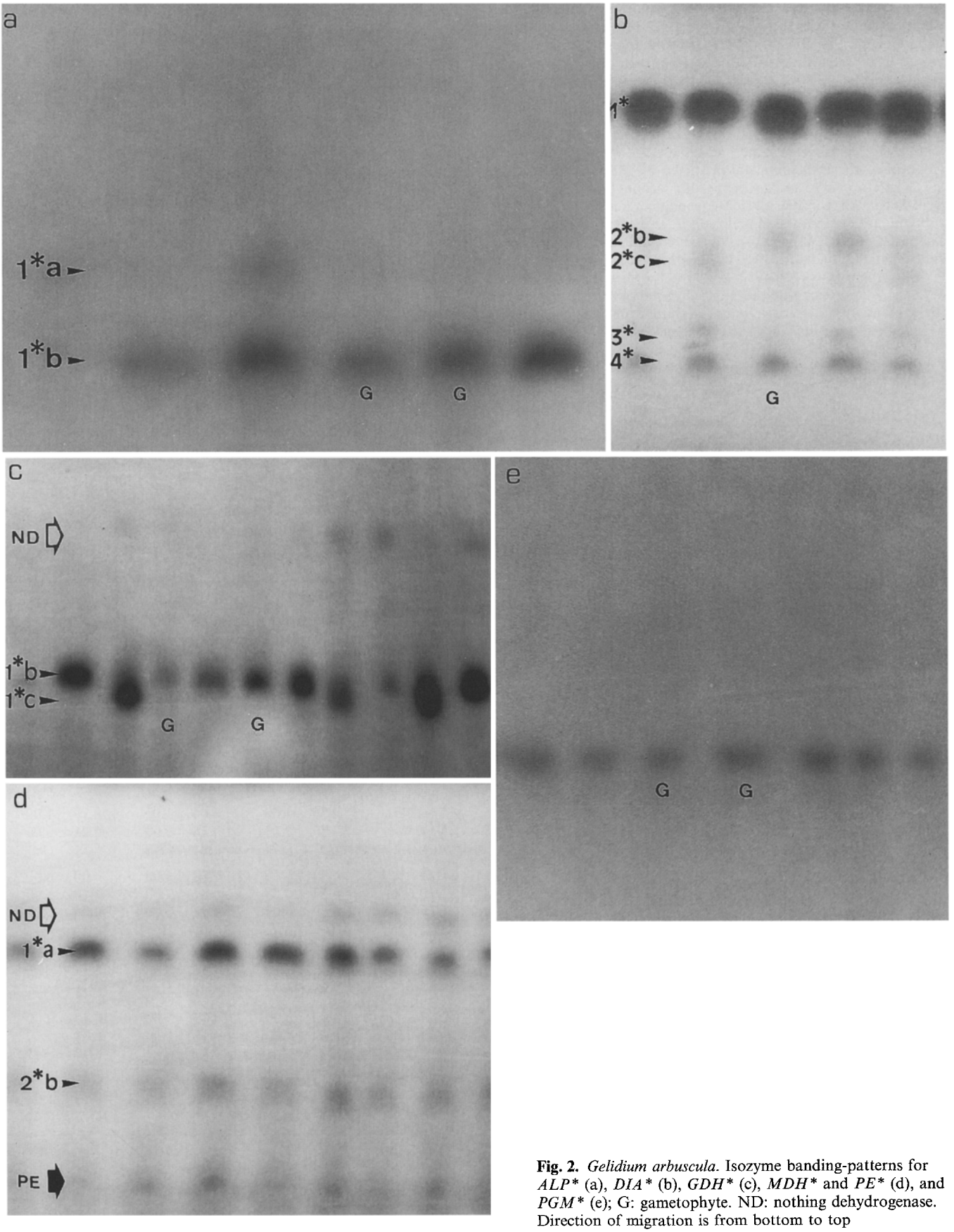

Fig. 2. *Gelidium arbuscula.* Isozyme banding-patterns for *ALP** (a), *DIA** (b), *GDH** (c), *MDH** and *PE** (d), and *PGM** (e); G: gametophyte. ND: nothing dehydrogenase. Direction of migration is from bottom to top

Table 3. *Gelidium arbuscula.* Number of alleles (*n*), proportion of loci polymorphic (P), average number of alleles per locus (A/L), and average gene diversity (*H*), recorded in sporophytic (S) and gametophytic (G) subpopulations from three locations in Canary Islands

Location	(*n*)	A/L	P	*H*
Gáldar				
S	(26)	1.18	0.136	0.070
G	(24)	1.09	0.091	0.021
Agaete				
S	(29)	1.32	0.273	0.065
G	(23)	1.04	0.045	0.018
Puerto Cruz				
S	(27)	1.23	0.227	0.074
G	(23)	1.04	0.045	0.015
$\bar{x}$ S	(27)	1.24	0.212	0.070
$\bar{x}$ G	(23)	1.06	0.060	0.018
$\bar{x}$ S+G	(25)	1.15	0.136	0.044

Table 4. *Gelidium arbuscula.* Observed, O, and expected (E) genotypes for each polymorphic loci at three locations studied

Genotype	Gáldar		Agaete		Puerto Cruz	
	O	(E)	O	(E)	O	(E)
ALP-1 a/a*	0	(0)	3	(0)	0	(0)
ALP-1 b/b*	42	(42)	43	(49)	38	(38)
ALP-1 a/b*	0	(0)	3	(0)	0	(0)
DIA-2 a/a*	0	(0)	0	(0)	20	(29)
DIA-2 b/b*	6	(0.3)	36	(25.5)	2	(2)
DIA-2 c/c*	14	(25)	0	(3.4)	0	(0)
DIA-2 a/b*	0	(0)	3	(0)	24	(15)
DIA-2 a/c*	0	(0)	0	(0)	0	(0)
DIA-2 b/c*	11	(5.7)	9	(19.1)	0	(0)
EST-2 a/a*	6	(45)	11	(38)	18	(31)
EST-2 b/b*	6	(0)	0	(0)	0	(0)
EST-2 a/b*	33	(0)	27	(0)	13	(0)
GDH-1 a/a*	6	(1)	0	(0)	0	(0)
GDH-1 b/b*	9	(23)	33	(39)	35	(35)
GDH-1 c/c*	2	(0)	4	(0)	0	(0)
GDH-1 a/b*	14	(9.4)	0	(0)	0	(0)
GDH-1 a/c*	2	(0)	2	(0)	0	(0)
GDH-1 b/c*	0	(0)	0	(0)	0	(0)
MDH-1 a/a*	46	(46)	32	(48)	12	(31)
MDH-1 b/b*	0	(0)	4	(0)	0	(0)
MDH-1 a/b*	0	(0)	12	(0)	19	(0)
MDH-2 a/a*	0	(0)	0	(0)	0	(0)
MDH-2 b/b*	43	(43)	46	(48)	19	(31)
MDH-2 a/b*	0	(0)	2	(0)	12	(0)
PGI-3 a/a*	0	(0)	0	(0)	0	(0)
PGI-3 b/b*	39	(39)	49	(49)	32	(37)
PGI-3 a/b*	0	(0)	0	(0)	5	(0)

Table 4 compares the observed and expected numbers of each genotype for each locality. As <1 degree of freedom was recorded for each polymorphic locus, no chi-square test could be performed, but important differences were nevertheless apparent. Although *EST-2*a/a* was the predominant genotype expected for all localities, *EST-2*a/b* was the predominant genotype observed. Similarly, *DIA-2** exhibited clear differences in observed and expected genotypes for all localities. The genotype *GDH-1*b/b* was expected to be predominant in Gáldar samples, but *GDH-1*a/b* was the more predominant genotype observed. Similar results were obtained for *MDI-1** and *MDH-2** loci from Puerto Cruz. In most cases, the allele fixation in gametophytic subpopulations was higher than in sporophytic subpopulations.

Table 5. *Gelidium arbuscula.* Chi-square comparison of allelic frequencies in sporophytic and gametophytic subpopulations from same locality in Canary Islands. Values are means (degrees of freedom) −: no test possible (invariable locus)

Locus	Locality		
	Gáldar	Agaete	Puerto Cruz
*ALP-1**	–	3.1 (1)	–
*DIA-2**	7.3 (1)**	8.8 (2)*	1.1 (1)
*EST-2**	19.8 (1)***	14.3 (1)***	7.8 (1)**
*GDH-1**	7.9 (2)*	4.3 (1)*	–
*MDH-1**	–	7.0 (1)**	10.3 (1)***
*MDH-2**	–	0.7 (1)	4.9 (1)*
*PGI-3**	–	–	2.1 (1)
Total	35.0 (4)***	38.2 (7)***	26.2 (5)***

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 6. *Gelidium arbuscula.* Nei's genetic distance, *D* (above diagonal) and genetic identity, *I* (below diagonal) (Nei 1972), between sporophytic (S) and gametophytic (G) subpopulations from Canary Islands. $D = 0.028 \pm 0.011$, $D = 0.030 \pm 0.009$, $D = 0.026 \pm 0.007$, and $D = 0.028 \pm 0.003$, between sporophytic plus gametophytic subpopulations, between sporophytes, between gametophytes, and between sporophytic and gametophytic subpopulations, respectively

	Gáldar (S)	Gáldar (G)	Agaete (S)	Agaete (G)	Puerto Cruz (S)	Puerto Cruz (G)
Gáldar (S)		0.019	0.024	0.027	0.043	0.047
Gáldar (G)	0.981		0.040	0.019	0.043	0.036
Agaete (S)	0.976	0.961		0.010	0.024	0.035
Agaete (G)	0.973	0.981	0.990		0.026	0.023
Puerto Cruz (S)	0.958	0.958	0.976	0.974		0.009
Puerto Cruz (G)	0.954	0.965	0.966	0.977	0.991	

Table 5 shows the results of chi-square tests testing for differences in allelic frequency at each polymorphic locus. Most comparisons between independent loci revealed significant differences at each locus, and χ^2 tests were, as a whole, significant at the 0.001% level for all comparisons.

Table 6 shows Nei's genetic identity, *I* (below diagonal), and genetic distance, *D* (above diagonal), between subpopulations of *Gelidium arbuscula.* The average genetic distance between all subpopulations was 0.028, while the average genetic distance between sporophytic subpopulations was 0.030, and between gametophytic subpopulations 0.026.

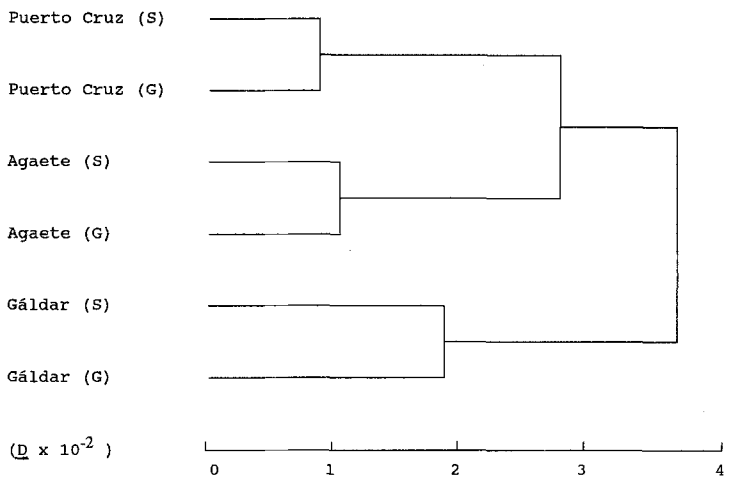

Fig. 3. *Gelidium arbuscula.* UPGMA dendrogram of genetic distances, *D* (Nei 1972), between sporophytic (S) and gametophytic (G) subpopulations from the Canary Islands

Table 7. *Gelidium arbuscula.* Coefficient of genetic differentiation (Nei 1973) between all sporophytic and gametophytic subpopulations for each polymorphic loci. G_{ST}: measure of amount of differentiation among subpopulations (indicates genetic differentiation between alleles of different populations)

Sporophytic subpopulations		Gametophytic subpopulations	
Locus	G_{ST}	Locus	G_{ST}
*ALP-1**	0.062	*GDH-1**	0.120
*DIA-2**	0.404	*DIA-2**	0.548
*EST-2**	0.061	$\bar{x}$	0.334
*GDH-1**	0.227		
*MDH-1**	0.116		
*MDH-2**	0.112		
*PGI-3**	0.048		
$\bar{x}$	0.147		

Fig. 3 is an UPGMA dendrogram of genetic distances, *D*, between subpopulations. Subpopulations of the same locality (independent of their ploidy) displayed the least genetic distance. No relationship was found between genetic and geographic distance. Subpopulations from different islands (Agaete and Puerto Cruz, Fig. 1) exhibited less genetic distance than subpopulations from the same island (Agaete and Gáldar, Fig. 1).

Table 7 shows the coefficient of genetic differentiation for the individual polymorphic loci, and the average differentiation between sporophytic subpopulations, and that between gametophytic subpopulations. The degree of differentiation was higher between haploid subpopulations ($G_{ST} = 0.334$) than between sporophytic subpopulations ($G_{ST} = 0.147$).

Discussion

This study on *Gelidium arbuscula* is the first to compare allelic frequencies between sporophytic and gametophytic subpopulations of a seaweed. Differences in allelic frequencies within each locality (Tables 2 and 5) and departures from Hardy-Weinberg equilibrium in sporophytic subpopulations (Table 4) indicate significant differences among the gene pool of sporophytic and gametophytic subpopulations. Sporophytes and gametophytes of *G. arbuscula* coinhabit, with no along-shore differences. Although we cannot dismiss the possible existence of selective forces acting in each subpopulation to maintain a given gene frequency, the predominance of asexual reproduction in both states could be the main reason of the genetic differentiation within each locality. Stoloniferous outgrowths of creeping axes is a common way for the propagation of *Gelidium* spp. on hard substrate (Oliveira 1989, Rueness and Fredriksen 1989, Santelices 1989, 1990, Gorostiaga 1990). Vegetative propagation has been interpreted as a conservative mechanism of adaptation in seaweeds (Stebbins and Hill 1980, Santelices 1990). When populations are able to reproduce asexually for several years, Hardy-Weinberg deviations and non-random associations of genotypes develop (Black and Johnson 1979, Hebert and Crease 1983, Innes and Yarish 1984, Gallagher 1986, Innes 1987, 1988). A predominance of vegetative propagation will decrease the genetic flow between sporophytes and gametophytes, increasing the differences between gene pools of both within the same population. Innes and Yarish (1984) described large deviations from expected genotypes for populations of sexually reproducing individuals in five populations of *Enteromorpha linza* (L.) J. Ag. from Long Island, USA. These deviations were attributed to the demonstrated asexual reproduction of *E. linza*, with no evidence of segregation by laboratory-culture experiments (Innes and Yarish 1984). Cheney and Babbel (1978) described heterozygote deficiences and Hardy-Weinberg deviations in two ACPH loci of *Eucheuma nudum*, which exhibited a propensity for vegetative reproduction (Cheney and Babbel 1978). Malinowsky (1974) has suggested asexual reproduction to be the cause of the high rate of fixed heterozygotes detected in populations of *Codium fragile* and

the inherent deviations of expected genotypes (Malinowsky 1974).

Gelidium arbuscula is genetically depauperate in the three populations studied. Gametophytic subpopulations have the lowest genetic variability described for seaweeds (Table 8), with the exception of cultivated Japanese populations of *Porphyra yezoensis* (Miura et al. 1979). The cultivated strains of *P. yezoensis* have been subjected to selection over a period of years, which is probably the cause of their low level of genetic variability. The genetic variability of the sporophytic subpopulations of *G. arbuscula*, although higher than that of the gametophytic subpopulations, is also the lowest described for diploid populations of seaweeds (Table 8).

Gelidium spp. are slow-growing perennial rodophytes, which undergo very few changes in biomass, and regenerate new individuals from an established holdfast (Silverthorne 1977, Akatsuka 1986, Oliveira 1989, Santelices 1989, 1990, Gorostiaga 1990). A recent "bottleneck" is therefore unlikely to be the cause of the low levels of genetic variation observed in these populations; were most of the available substrate occupied by perennial individuals with long life-spans, then recruitment would be severely limited and the genetic composition of a given year would remain relatively stable (Innes 1987). Their location – volcanic islands – suggests that these populations may have arisen through a founder effect. This, combined with predominantly asexual propagation, could explain the low genetic variability in *G. arbuscula* from the Canary Islands. It has been reported that asexual reproduction can result in reduced genetic variability in a population by magnifying the effects of selection, random-drift or founder effects (Black and Johnson 1979, Innes and Yarish 1984). In fact, of the factors determining the genetic structure of a population, none has a more profound effect than the mode of "breeding strategy", with the degree of allozyme variation being a strong indicator of the type of breeding strategy employed by a species (Selander and Ochman 1983, Baur and Klemm 1989). The effects of genetic drift arising from founder events have been estimated to result in a 65% reduction in heterozygosity (Nei et al. 1975) and a simultaneous important reduction in the average number of alleles per locus (Systma and Schaal 1985). This appears to be the case for *G. arbuscula* from the Canary Islands. A similar interpretation has been made for natural populations of *Porphyra yezoensis*, in which selfing and asexual propagation have been hypothesized to be responsible for the low genetic variability and the high number of fixed alleles detected (Fujio et al. 1985, 1987), and similar results have been reported for other selfing organisms (Selander and Hudson 1976, Systma and Schaal 1985, Soltis and Soltis 1987, Baur and Klemm 1989).

Table 8. *Gelidium arbuscula*. Comparison of genetic variability of natural populations. A/L: Average number of alleles per locus; P: average proportion of polymorphic loci. *H*: Average gene diversity. (h): haploid individuals. (d): diploid individuals. (h, d): haploid and diploid individuals. pop.: populations; sub.: subpopulations; –: no data

Species	A/L	P	*H*	Source
Codium fragile (d)	–	0.310	0.150	Malinowsky (1974)
Eucheuma nudum (h, d)	1.31	0.313	–	Cheney and Babbel (1978)
E. isiforme (h, d)	1.36	0.364	–	
E. gelidium (H, d)	1.29	0.286	–	
E. acanthocladum (h, d)	1.25	0.250	–	
Porphyra yezoensis (h)	1.64	0.333	0.127	Fujio et al. (1985)
P. yezoensis (h)	–	0.583	0.197	Fujio et al. (1987)
P. yezoensis (h)				
Wild pop.	1.50	0.398	–	Miura et al. (1979)
Cultured pop.	1.00	0.000	–	
Gelidium arbuscula				
Sporophytes sub. (d)	1.24	0.212	0.070	Present study
Gametophytes sub. (h)	1.06	0.060	0.018	

In the present study, analysis of phylogenetic relations between subpopulations by the UPGMA method has revealed the absence of a correlation between genetic and geographic distances, and the existence of a higher genetic identity between subpopulations at the same locality (independent of the ploidy of the individuals). This indicates that, in addition to high genetic differentiation between subpopulations at the same locality, genetic flow between localities is very reduced or non-existent. *Gelidium* spp. have been described as a genus which release spores, and free-floating fragments of this seaweeds do not seem to be very successful in establishing new populations (Coon et al. 1972, Alvarez et al. 1978, Amsler and Searles 1980, Okuda and Neushul 1981, Gorostiaga 1990, Santelices 1990), supporting the high values for the coefficient of differentiation detected between all localities in the present study (Table 7).

The coefficient of differentiation between localities was higher between gametophytic subpopulations (G_{ST} = 0.334) than between sporophytic subpopulations (G_{ST} = 0.147). Similar results have been reported for wild populations of the haploid laver *Porphyra yezoensis*, which had similar and even higher differentiation coefficients (0.623, 0.125) between populations only a few meters apart (Fujio et al. 1985, 1987). High genetic differentiation has even been described in areas separated by only a few hundred meters for *Enteromorpha linza*, suggesting that geographic microdifferentiation plays some role in generating such differences (Innes 1987, 1988).

It is difficult to explain the low genetic variability and the genetic structure of populations of *Gelidium arbuscula* from the Canary Islands. An increased founder effect, due to a lower number of gametophytes than sporophytes during the colonizing process, combined with predominant asexual reproduction would magnify genetic differentiation, fixation of alleles, and lower the genetic variation of gametophytes. If the same capacity for reproduction and recruitment is assumed for both sporophytes and gametophytes, then the population structure of *G. arbuscula* would be the consequence of the colonizer step; i.e., when populations originated. In the three populations analyzed, the number of mature gametophytic individuals detected was always lower than the number of mature sporophytes (S:G = 7:1; own unpublished data), independent of season or sampling locality. A predomi-

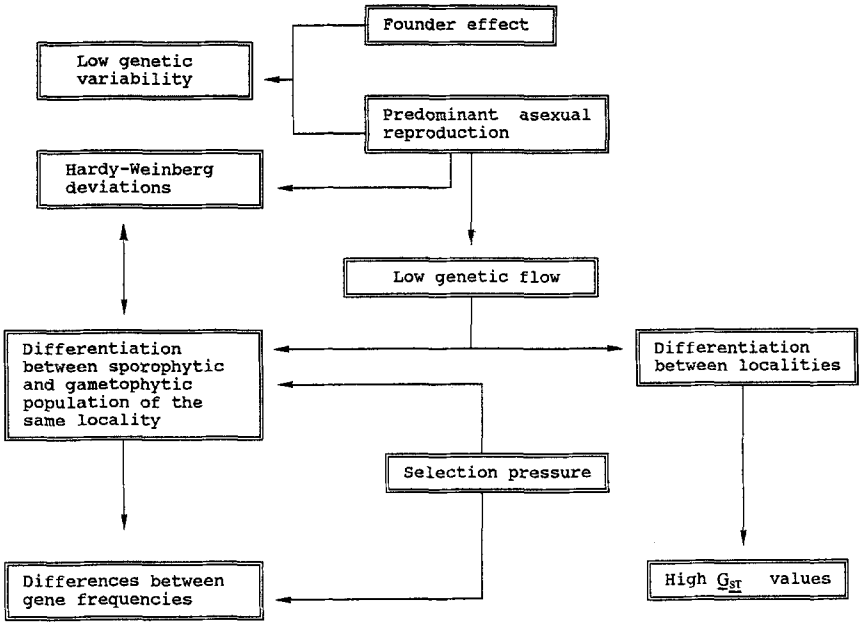

Fig. 4. *Gelidium arbuscula*. Schematic of genetic structure and genetic differentiation of the Canary Islands populations

nance of sporophytes has been described for several populations of *G. sesquipedale* (Seoane-Camba 1965, 1969, Gorostiaga 1990), *G. robustum* (Guzmán del Proó and de la Campa de Guzmán 1969), *G. pristoides* (Carter 1985, Robertson et al. 1985), and *G. amansii* (Akatsuka 1986). This disproportion supports the idea that alternating generations and consequent sexual reproduction is not as important as other ways of propagation.

On the other hand, the higher genetic variability of isozymes recorded for the sporophytic subpopulations of *Gelidium arbuscula* could reflect a higher variability of the global diploid genome, which could promote higher adaptation of diploids and explain the predominance of sporophytes in the populations. Evidence that natural selection could directly or indirectly influence the allele frequencies is suggested from the observations of significant differences in allelic frequency between sporophytes and gametophytes at one and the same locality.

Although it is difficult to estimate fitness differentials associated with loci coding for enzymes, particularly in natural populations (Hilbish and Koehn 1985), genetic factors have been hypothesized to be involved in the predominance of the diploid phase in the life-history of seaweeds (Hansen and Doyle 1976, Litler et al. 1987). Relationships between the polymorphic loci of isozymes and fitness or differentiation processes have been reported. Innes (1988) described significant differentiation between high and low intertidal positions with respect to the *GOT-2* locus in *Enteromorpha linza*. One of the clones (Clone 6, *GOT-2* FM) was usually associated with the high intertidal zone. Miura et al. (1978 b) reported a relationship between the catalase genotype and morphology in *Porphyra yezoensis*. Those thalli with the CAT^A gene were wider and had smooth edges, whilst those thalli with the CAT^B gene were narrower and had crinkled edges. Okumura and Fujino (1986) described a close relationship between the water temperature of the habitat and frequencies of thermostable alleles at the PGM, PGI and GDH loci in two populations of *P. yezoensis*, suggesting that these three loci act as a polygenic system that contribute to temperature tolerance of the seaweed. For the marine mussel *Mytilis edulis*, Hilbish and Koehn (1985) described differences in catalytic efficiences of the LAP locus, and suggested that an allele-frequency cline operates by natural selection at this locus. Similar results have been reported for *Crassostrea virginica* (Singh and Zourous 1978, Zouros et al. 1980) and for *Drosophila* spp. (reviewed by Lewontin 1985).

Fig. 4 summarizes the events that could explain the genetic differentiation and genetic structure of the populations of *Gelidium arbuscula* in the Canary Islands.

Additional studies are needed to determine the importance of ploidy to the relative fitness of sporophytic and gametophytic subpopulations of isomorphic species, but the interaction of drift with natural selection could explain the genetic structure seen in *Gelidium arbuscula*.

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