

GENETIC STRUCTURE OF NATURAL POPULATIONS IN THE RED ALGAE *GELIDIUM CANARIENSE* (GELIDIALES, RHODOPHYTA) INVESTIGATED BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS¹

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Random amplified polymorphic DNA (RAPD) marker variation was analyzed in female gametophytes in natural populations of *Gelidium canariense* (Grunow) Seoane-Camba ex Haroun, Gil-Rodríguez, Diaz de Castro et Prud'Homme van Reine from the Canary Islands to estimate the degree and distribution of genetic variability and differentiation. A total of 190 haploid individuals were analyzed with 60 polymorphic RAPDs bands which produced 190 distinct multilocus genotypes. A high level of polymorphism was detected in all populations analyzed. Within-population gene diversity ranged from 0.156 to 0.264. The populations on the island of Gran Canaria showed higher genetic variation than the other populations analyzed. The partitioning of molecular variance by analysis of molecular variance showed that most genetic variation resides within populations (68.85%). These results suggest that sexual reproduction is the predominant mode of reproduction for *G. canariense* gametophytic populations, and the main determinant in reaching high levels of genetic diversity. The Neighbor-Joining tree and FCA analysis displayed two subclusters that correspond to the populations from the western islands (Tenerife, La Palma, Gomera) and the eastern island (Gran Canaria). In addition, we have detected a significant relationship between $F_{ST}/(1 - F_{ST})$ and geographical distance consistent with data on water circulation and age of islands. The results obtained agree with an isolation by distance model, with gene flow from eastern to the western islands, and a high level of genetic differentiation between populations ($F_{ST} = 0.311$, $P < 0.001$).

Key index words: Canary Islands; *Gelidium canariense*; genetic diversity; genetic structure; population genetics; RAPD; Rhodophyta

Abbreviations: AMOVA, analysis of molecular variance; FCA, factorial correspondence analysis; IBD, isolation by distance; RAPD, random amplified polymorphic DNA

Understanding the spatial genetic structure of populations, i.e. the degree and organization of genetic variation in space, is an important task in evolutionary genetics. As a rule, the degree to which organisms interchange genetic diversity is regulated by the levels of gene flow among locally breeding populations through dispersal corridors and/or suboptimal habitats (Frankham 1996, Frankham et al. 2002). When genetic variation is structured in space, several theoretical and empirical reports suggest that reproductive performance and survival against stochastic events are greatly improved (Huenneke 1991, Frankham 1996, Caujapé-Castells and Pedrola-Monfort 1997, Frankham et al. 2002). Understanding patterns of genetic variation and population structure within a species is, therefore, crucial in studies of ecology and evolution, because it permits an insight into the transmission of genes from generation to generation, and a prediction of chances of long-term survival and the continued evolution of populations and species (Sosa et al. 2002). In this sense, molecular markers are an important tool in understanding and predicting the mechanisms that cause interpopulational genetic differentiation.

Although allozymes have been successfully used to examine genetic structure for many organisms, they generally provide few loci and low levels of polymorphism in seaweeds (Sosa and Lindstrom 1999, Valero et al. 2001). In this context, a diverse array of

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molecular tools has been developed in several algal species for high-resolution population-level genetic studies. Especially important has been the development and growth in macroalgae of studies using microsatellites (Billot 1999, Van der Strate et al. 2000, 2003, Engel et al. 2004, Billard et al. 2005, Guillemain et al. 2005). However, we should not underestimate the use and importance of other molecular techniques such as random amplified polymorphic DNAs (RAPDs) (Coyer et al. 1997, Miller et al. 2000, Wright et al. 2000, Faugeron et al. 2001, Meneses 2001, Martínez et al. 2003, Faugeron et al. 2004). The advantages and disadvantages of RAPD analysis have been widely debated (Welsh and McClelland 1990, Williams et al. 1990, Hadrys et al. 1992, Sunnucks 2000). Although some authors (Van Oppen et al. 1996, Coyer et al. 1997) dismiss the use of RAPDs for small-scale geographical studies, the general rule is that when a large number of markers are included and the analysis is restricted to taxa in which band homology assessments can be confidently made (conspecific populations), the results of RAPD assessments have been successfully incorporated into evaluations of inter- and intrapopulation differentiation of natural populations (Waycott 1998, Bartish et al. 1999, Hsiao and Lee 1999, Bouza 2002, Bouza et al. 2002, González-Pérez et al. 2004). One of the main disadvantages of RAPD markers is their dominant character. However, in those organisms that show a haploid-diploid life cycle with a haploid isomorphic stage (like *Gelidium*), dominance of RAPD markers can be avoided by selecting haploid individuals.

Gelidium canariense (Grunow) Seoane-Camba ex Haroun, Gil-Rodríguez, Diaz de Castro et Prud'Homme van Reine is a benthic red seaweed distributed along the coasts of the Canary Islands. *Gelidium canariense* grows in clumps on wave-exposed northern rocky shores in the lower intertidal region and typically exhibits a triphasic isomorphic life cycle (Sosa 1991, Sosa and Garcia-Reina 1993, Sosa et al. 1993, 1998, Lindgren et al. 1998). *Gelidium canariense* is an endemic from the Canary Islands which is distributed almost exclusively in the north and northeast coast of the most western islands (Sosa and Garcia-Reina 1993). This species shows a system of erect fronds which develop from a system of small, intermingled and ramified prostrate axis, and the general structure of its populations is highly stable indicating a high survival rate of clumps (Lindgren et al. 1998).

Either of the life-cycle phases of the species (gametophytes and tetrasporophytes) can dominate in natural populations (Sosa and García-Reina 1993, Lindgren et al. 1998), and, so far, no significant genetic, physiological or demographic differences have been noticed between the phases (Sosa et al. 1993, 1998, Lindgren et al. 1998).

From a genetic point of view, natural populations of *G. canariense* have been studied by allozyme electrophoresis (Sosa and García-Reina 1993). From the low genetic diversity detected and the existence of differ-

ences between gametophytic and tetrasporophytic allelic frequencies it was concluded that asexual reproduction constituted the predominant form of reproduction in the analyzed populations (Sosa and García-Reina 1993). However, due to the low number of gametophytic individuals analyzed, a possible bias could have been present in this study. Thus, Sosa et al. (1998) re-evaluated the data using only the diploid gene frequencies. That study proved that *G. canariense* populations showed no significant differences in allelic frequencies among tetrasporophytic and gametophytic subpopulations with all the populations in Hardy-Weinberg equilibrium, showing the importance of sexual reproduction in this species. In addition, significant genetic differentiation among populations separated 30–100 km, was detected, with a mean F_{ST} value of 0.130 among all populations analyzed, indicating a low level of gene flow between populations (Sosa et al. 1998). These authors suggested that long distance dispersal seemed to be insufficient to prevent genetic differentiation among populations.

Our objectives in this paper are to use information provided by RAPD polymorphisms to gain insight into: (i) the amounts and spatial structuring of genetic variability in populations of *Gelidium canariense* by analyzing haploid female individuals, (ii) the contribution of the reproductive strategy in the maintenance of genetic variation, and (iii) the role of geographical isolation on its interpopulation genetic differentiation.

MATERIALS AND METHODS

Sampling design. A total of 190 fertile female gametophytic fronds, identified by the presence of cystocarps, belonging to seven natural populations of *G. canariense* in the Canary Islands were analyzed (Fig. 1). This sampling design is a thorough representation of the geographical distribution of this organism in the Canaries. About 30 individuals were collected from each population, except for Hermigua (Gomera) where only 12 individuals were found. The within-population sampling area ranged between 4 and 10 m². To make sure that each individual sample corresponded to a single specimen, only tufts physically separated were chosen, and only one frond from each tuft was selected for analysis. Once in the laboratory, samples were examined carefully to select epiphyte-free gametophytic fronds for DNA extraction. The apical portions of the chosen branches were cut, washed with 1% SDS in distilled water and then blotted dry. Each sample was preserved with silica gel in the dark until used.

DNA extraction. About 200 mg (dry weight) of algal material was used for DNA extraction. Algal tissue was ground in liquid nitrogen in a sterile mortar with a pestle, and the resulting powder was transferred to a 2 mL tube. Total genomic DNA was extracted following Cenis (1992) modifications for seaweeds following Alberto et al. (1999). All the raw DNAs were dissolved in 50 µL of TE buffer and further purified using the Wizard™ DNA Clean-Up System (Promega Biotech, Waltham, MA, USA). The presence of degraded DNA and the concentrations of the DNA samples were tested by comparison with known quantities of pre-purified calf thymus DNA (Pharmacia Biotech, Uppsala, Sweden) (Sambrook et al. 1989) in a 1% agarose gel. Yield was approximately of 0.10 µg/mg.

RAPD amplification. The individuals from each population were tested in an initial screening with 80 10-mer primers

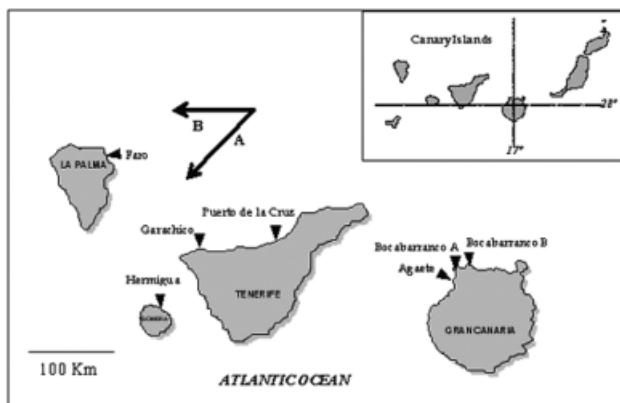


FIG. 1. Map of the geographical distribution of the sampled populations showing the direction of general water current (arrow A) and surface water current (arrow B) in the Canary Islands.

(Operon Technologies Inc., Huntsville, AL, USA. Kits K, L, M, N) for their ability to generate reproducible amplification products. For each individual and each primer, duplicate reactions and reactions with DNAs extracted independently from the same individual were used. Final reaction mixtures contained between 25 and 30 ng of genomic DNA and a mastermix, $1 \times$ PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 160 μ M of each dNTP, 1.5 mM MgCl₂, 1 unit Taq DNA polymerase buffer (Perkin-Elmer, Boston, MA, USA) and 0.4 μ M of the primers. Amplifications were run in a Perkin-Elmer Gene Amp PCR System 2400 and gave optimal bands with an initial denaturation step of 94° C for 1.5 min 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. The concentration of DNA used was found to be optimal with respect to reproducibility and minimization of secondary ghost banding (technique background). Reaction mixes without DNA were run as blanks in all RAPD amplifications. Eight primers gave optimally reproducible polymorphic bands and were thereby selected for further analysis. Three samples had to be excluded from the final batch of amplifications because of DNA degradation, leaving a total of 190 plants (Table 1). Amplification products were resolved by 1.8% agarose gel electrophoresis in TBE buffer (45 mM Tris-Borate and 1 mM EDTA) and visualized under ultraviolet light after ethidium bromide staining. Gels were photographed using a digital camera Kodak DC40 (Kodak SA, Madrid, Spain). Scoring was carried out conservatively, excluding unreliable bands (faint and non-reproducible bands). All polymorphisms whose intensities did not allow a straightforward interpretation were re-checked by repeating the amplification under the same conditions.

Data analysis. Bands that were present at a frequency lower than 0.05 in the whole sample were not considered for this analysis. Each band scored was considered as a locus with two alleles ("present" and "absent") and the allelic frequencies could be directly determined. Two types of data analyses were performed. The first considered each locus separately and used the classical gene frequency approach (monolocus data analysis). The second analysis treated the individual banding profile as a multilocus genotype and used genotype frequencies (multilocus data analysis).

Monolocus data analysis. For each population, allele frequency and gene diversity, measured as the expected heterozygosity (H) averaged over loci (Nei 1978), were calculated using the FSTAT version 2.9 program (Goudet 1999). Polymorphism was calculated with GENETIX pack-

age version 4.02 (Belkir et al. 1996). Gene frequencies were analyzed using a nested analysis of variance (analysis of molecular variance [AMOVA], Excoffier et al. 1992), calculated using ARLEQUIN ver. 2.0 software (Schneider et al. 2000), to estimate the components of variance among and within islands and populations from Gran Canaria and Tenerife.

We applied AMOVA to assess the partitioning of RAPD variation in the gametophytic populations of *G. canariense* at different hierarchical levels and test for significance against the null hypothesis of no structure using ARLEQUIN ver. 2.0 software (Schneider et al. 2000).

To explore the patterns of interpopulation genetic differentiation in space, we carried out a Mantel test (Mantel 1967). These involved an interpopulation genetic similarity matrix based on $F_{ST}/(1 - F_{ST})$ and a geographic distance matrix. Significance for the Mantel statistic was calculated using 1000 random permutations with IBD software (Bohonak 2002).

Genetic distances between pair wise combinations of populations were expressed as $D = -\log(1 - F_{ST})$ (Reynolds et al. 1983). These values were calculated and used as the input for the construction of the interpopulation Neighbor-Joining with POPULATION software (Langella 2000), and graphically displayed with TREEVIEW (Page 1996).

Multilocus data analysis. Multilocus genotype diversity was examined to assess the importance of recombination. In species reproducing exclusively asexually, genotype diversity is the result of the combined effects of mutation and migration, whereas in outcrossing species recombination is the principal source of diversity. Numbers of unique and shared multilocus genotypes and of pairwise differences among genotypes within each population were counted using the ARLEQUIN package (Schneider et al. 2000).

A multivariate representation of the analyzed individuals was carried out by subjecting presence/absence of RAPD fragments to Factorial Correspondence Analysis (FCA) in GENETIX version 4.02 (Belkir et al. 1996).

RESULTS

Monolocus data analysis. A total of 60 polymorphic markers with eight primers were scored in all gametophytic populations, ranging in size from 250 to 1700 bp. Loci were identified by indicating first the name of the primer and then the size of the RAPD fragment (in base pairs). The mean percentage of polymorphic loci and gene diversity (H) was slightly higher in Gran Canaria island (average $P = 88\%$, average $H_e = 0.251$) than in the other islands (Table 1). The mean gene diversity (H averaged over stands) was 0.219 for all populations analyzed, ranging from 0.156 (PC) to 0.264 (BB). Similarly, values for gene diversity were lower in the western island (Tenerife, Gomera, and La Palma) populations than Gran Canaria populations (Table 1). Despite the high levels of variation detected, we did not find any monomorphic population-exclusive alleles. However, eight island-exclusive alleles were detected: seven in Gran Canaria and one in Tenerife. On the other hand, unexpectedly, there were two private alleles between Gran Canaria and Gomera Island, which were not present in Tenerife populations.

Overall, most of the RAPD variability was distributed within populations at all geographical levels examined (Table 2). Roughly 70% of the variation among all islands is explained by the intra-population compo-

TABLE 1. Number of individuals analyzed (*N*), percentage polymorphic loci ($P_{0.99}$), and gene diversity (*H*, unbiased genetic diversity, Nei 1978).

| Island | Population | Code | <i>N</i> | <i>P</i> | <i>H</i> |
|--------------|----------------|------|----------|----------|----------|
| Gran Canaria | Bocabarranco A | BA | 31 | 86.7 | 0.250 |
| Gran Canaria | Bocabarranco B | BB | 32 | 86.7 | 0.264 |
| Gran Canaria | Agaete | AG | 29 | 90.0 | 0.240 |
| Tenerife | Puerto La Cruz | PC | 27 | 55.0 | 0.156 |
| Tenerife | Garachico | GA | 29 | 70.0 | 0.211 |
| La Palma | Faro | FP | 30 | 68.3 | 0.200 |
| Gomera | Hermigua | GO | 12 | 58.3 | 0.210 |
| Average | | | 27 | 73.6 | 0.219 |

ment. The two variance components (among islands and among populations within islands), and their related fixation indices ($F_{CT} = 0.206$ and $F_{SC} = 0.133$, respectively, Table 2) were significant.

Global genetic differentiation among populations was highly significant ($F_{ST} = 0.311$; $P < 0.001$). In addition, the fixation indices between populations within Tenerife and Gran Canaria island were significant ($F_{ST} = 0.136$; $P < 0.001$ and $F_{ST} = 0.104$; $P < 0.001$, respectively). Consequently, the magnitude of the genetic differentiation measured among populations from different islands (separated by 52–232 km) was at least 2.3 times higher than that among populations from the same island (separated by 0.5–21.5 km). The AMOVA revealed that 86.37% and 89.58% of the genetic variance was found within populations in Gran Canaria and Tenerife, respectively (Table 2). On the other hand, percentage of variation among regions (20.56%) was almost twice as the percentage of variation among populations within regions (10.59%).

A significant positive correlation between $F_{ST}/(1 - F_{ST})$ and geographical distance was taken as evidence for isolation by distance (Slatkin 1993). In the regression that used F_{ST} values to infer gene flow, geographical distance among populations explained 57% of the variation in gene flow. Mantel's test substantiated these qualitative geographic relationships by resulting in significant correlation between both distances (geographical and genetic).

TABLE 2. Values of AMOVA partitioning of RAPD variability at the three hierarchical levels considered.

| Source of variation | df | Variance components | Percentage of variation | Fixation indices |
|----------------------------------|-----|---------------------|-------------------------|--------------------|
| Regions | | | | |
| Among regions | 3 | 2.015 | 20.56 | $F_{CT} = 0.206^a$ |
| Among populations within regions | 3 | 1.038 | 10.59 | $F_{SC} = 0.133^b$ |
| Within populations | 373 | 6.748 | 68.85 | |
| Total | | | | $F_{ST} = 0.311^b$ |
| Gran Canaria | | | | |
| Between populations | 2 | 1.206 | 13.63 | $F_{ST} = 0.136^b$ |
| Within populations | 181 | 7.646 | 86.37 | |
| Tenerife | | | | |
| Between populations | 1 | 0.6562 | 10.42 | $F_{ST} = 0.104^b$ |
| Within populations | 110 | 5.6410 | 89.58 | |

AMOVA, analysis of molecular variance; RAPD, random amplified polymorphic DNA; df, degrees of freedom.

^a $p < 0.01$

^b $p < 0.001$

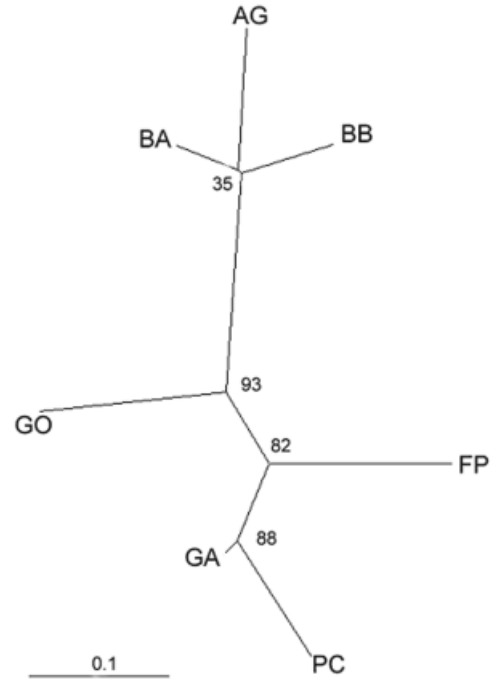


FIG. 2. Interpopulation Neighbor-Joining cluster based on Reynold's distances among the seven *Gelidium canariense* populations surveyed. Bootstrap values (based on 1000 permutations) are indicated in each node. Population codes correspond to those in Table 1.

The interpopulation grouping in the Neighbor-Joining tree based on Reynold's distances (Fig. 2, Table 3) is consistent with these results, as it clusters more tightly in populations from the same or adjacent islands.

Multilocus data analysis. The number of different genotypes was equal to the number of analyzed individuals; therefore, no identical multilocus genotypes of haploid individuals (haplotype) were detected within populations. The FCA based on multilocus RAPD genotypes summarized the inter-individual relationships in two multivariate components, which accounted for about 20% of the total

TABLE 3. Interpopulation genetic differentiation (F_{ST}) (above) between the seven populations of *Gelidium canariense* analyzed and geographic distances (km) between pairs of populations.

| | BA | BB | AG | PC | GA | FP | GO |
|----|-------|--------------|--------------|-------|--------------|-------|-------|
| BA | | <u>0.114</u> | <u>0.138</u> | 0.343 | 0.275 | 0.324 | 0.290 |
| BB | 0.5 | | <u>0.157</u> | 0.362 | 0.320 | 0.340 | 0.296 |
| AG | 12.0 | 12.5 | | 0.381 | 0.329 | 0.357 | 0.332 |
| PC | 116.8 | 117.3 | 116.2 | | <u>0.104</u> | 0.261 | 0.285 |
| GA | 137.4 | 137.9 | 137.6 | 21.4 | | 0.172 | 0.226 |
| FP | 231.2 | 231.7 | 232.5 | 128.7 | 112.5 | | 0.285 |
| GO | 168.2 | 168.7 | 156.2 | 71.4 | 52.0 | 93.7 | |

Underlined F_{ST} values correspond to populations from the same island.

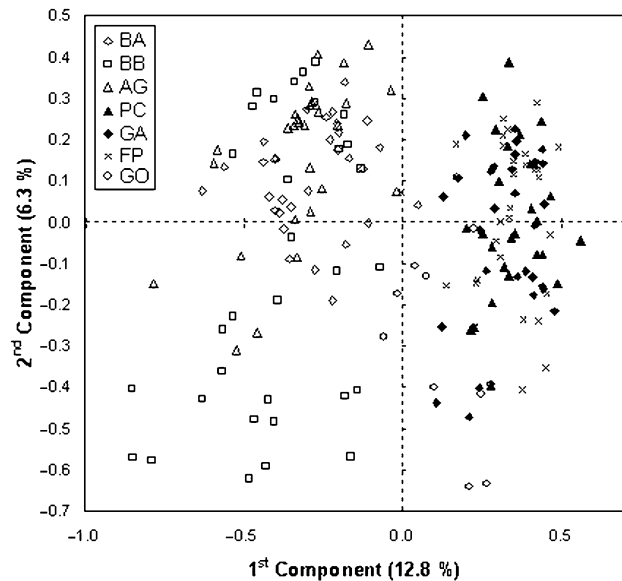


FIG. 3. Principal component analysis of *Gelidium canariense* populations. Percents in parentheses indicate the proportion of total variation explained by each component. Population codes correspond to those in Table 1.

variation (Fig. 3). The first FCA component separated the populations clearly into two distinct clouds that reflect the geographical localization of populations in the western islands (PC, GA, FP, GO) and in Gran Canaria (Fig. 3). However, some individuals from Gomera were much closer to those from Gran Canaria.

DISCUSSION

Narrow endemism and insularity are poor predictors of RAPD variability in the seven surveyed stands of *Gelidium canariense*. Unlike allozymes (Sosa and García-Reina 1993), RAPD detected high levels of gene diversity in all populations analyzed. Of all characteristics of an organism's life history, outcrossing and life forms in plants has been shown to have the most importance in determining levels of genetic variability. Populations of dioecious and perennial plants are typically character-

ized by high levels of within-population diversity. Our results fit with the top range of H values reported for sexually reproducing red seaweed species (Sosa and Lindstrom 1999, from allozyme data), and are similar to H values in gametophytes of the red alga *Mazzaella laminarioides* (Bory) Fredericq (Faugeron et al. 2001). Faugeron et al. (2004) found values of H higher than those detected for *G. canariense* in the obligate outcrossing red alga *Gigartina skottsbergii* Setchell et Gardner (ranging from 0.128 to 0.276).

The fact that no identical haplotypes were found for *Gelidium canariense* gametophytes constitutes such a surprising result that it could initially be attributed to inherent problems derived from the technique itself. However, since all fronds of *G. canariense* used for amplification were scrupulously cleaned, we rule out the possibility that some of the bands scored could be artefacts caused by amplification of epiphyte DNA. Furthermore, given that low complexity genomes (prokaryotes) have been unambiguously shown not to interfere with eukaryotic ones in RAPD amplifications (Williams et al. 1993), bacterial contamination can also be safely discarded as an influence on these results. Moreover, the previous trials of the technique were made using two replicas and all the cases obtained a very high percentage of reproducibility, varying between 89% and 100% among different amplifications (data not shown). Thus, high levels of gene diversity and no identical genotypes, in terms of RAPD markers, in *G. canariense* populations seems to be reflecting a real biological feature of this species, and are in concordance with the patterns expected for an outcrossing and moderately long-lived species.

Gelidium canariense has the potential to reproduce asexually, but (as discussed above) failure to find groups of identical individuals in the surveyed populations argues against a predominant action of this reproductive strategy. Faugeron et al. (2001) reported 70% distinct genotypes in haploids of the outcrossing red alga *Mazzaella laminarioides* with RAPDs. Faugeron et al. (2004) found 96% different genotypes in haploids of the rhodophyta *Gigartina skottsbergii* that only propagates through sexual reproduction. These values are very high compared with studies using multi-locus DNA markers for detecting genotypic variation in clonal (or highly selfing) species. Wright et al. (2000) reported 54% distinct RAPD phenotypes in the red apomitic seaweed *Delisea pulchra* (Greville) Montagne. The genetic composition in clonal and highly selfing species is mainly determined by the number of migrants, mutations, whereas in an outcrossing species, in addition to the stochastic migration events, distinct genotypes are regularly produced by recombination (Faugeron et al. 2001). We also consider that in *G. canariense*, recombination is more effective than immigration in producing different multi-locus genotypes. Therefore, our results suggest that sexual reproduction is the predominant reproductive mode in *Gelidium canariense*, and the main determinant in reaching high levels of genetic variability. This result agrees with those

obtained in other Canarian endemic terrestrial species which, despite presenting a restricted distribution, show high levels of genetic diversity. (Francisco-Ortega et al. 2000, Sosa et al. 2002).

Although only female gametophytes were analyzed, we consider the phases of *Gelidium canariense* to be completely isomorphic. They all grow in the same habitats without any spatial differentiation amongst them (Lindgren et al. 1998) and no physiological (Sosa et al. 1993), demographic (Lindgren et al. 1998) or genetic differences have been noted (Sosa et al. 1998). We therefore consider that it is feasible to extrapolate the results obtained in the female gametophytic phase to all the phases of the species.

As shown by the AMOVA, most of the high levels of RAPD variation in *G. canariense* are concentrated at the intrapopulation level. In outcrossing plant species, a large percentage of variation is commonly accounted for by differences among individuals within populations (Hamrick and Godt 1989, Bartish et al. 1999). Similarly, Van Oppen et al. (1995) interpret high levels of intra-population RAPD variation for the benthic alga *Phycodrys rubens* (Linné) Batters by a combination of drift and disruptive selection. In addition, Van der Strate et al. (2003) detected that most genetic variation (75%) in *Cladophoropsis membranacea* (Hofman Bang ex C. Agardh) Børgesen (Chlorophyta) in the Canary Islands occurred within sites.

In *Gelidium canariense*, there are several observations that suggest that the observed pattern is more likely the result of continuous disturbance provoked by the action of local hydrodynamics than of selection. First, our observations in the field do not support the existence of microhabitat differences. Populations of *G. canariense* always live in rocky beds very exposed to the action of waves. This is bound to cause considerable within-population ecological uniformity and might provoke random dispersal of genetically related individuals.

Although the major proportion of the total genetic variation of *G. canariense* was found among individuals within populations, F_{ST} values between population pairs also revealed significant interpopulation differentiation. Moreover, mean F_{ST} values among populations within islands are lower than those obtained among islands, and interpopulation dispersal in *G. canariense* seems to be a local phenomenon. Restricted gene flow (genetic isolation) probably results from the limited long distance dispersal of spores, gametes, or drifting reproductive fragments. Seaweeds are generally considered poor dispersers because spore survival is generally limited to a few days (Santelices 1990, Sosa and Garcia-Reina 1993). Reduced gene flow has been confirmed by indirect estimates for several species of algae (Engel et al. 1997, Wright et al. 2000, Faugeron et al. 2001, 2004, Zuccarello et al. 2001, Van der Strate et al. 2003), where genetic differentiation has been detected at distances shorter than 1 km. Faugeron et al. (2004) explain the reduced flow among closed populations of red alga *Gigartina skostteri* by local genetic drift exacerbated by its reduced dispersal abilities at

distances higher than 45 km. Our results can be interpreted in the same way.

Similarly, there was significant pattern of isolation by distance ($Z = 1116.7$, $r = 0.756$, $P < 0.01$) when comparing genetic distance and geographic distance, whereas the Neighbor-Joining tree (Fig. 2) and the FCA graph (Fig. 3) show a greater relationship between Tenerife, Gomera, and La Palma than between Gran Canaria and the other islands. Therefore, Gran Canaria seems to act as a donor source in the IBD model.

General current flow through the Canarian archipelago is usually Southwest (Llinás et al. 1994); however, surface currents caused by predominant northeast winds and the constant deflection of the direction of the superficial flow throughout the year from the continent toward the most western islands (Villagarcía et al. 1999), would favor the migration of individuals from the easternmost island (Gran Canaria) toward the westernmost ones (Tenerife, La Palma, and Gomera) (Fig. 1). However, Tenerife is a physical barrier that clearly disrupts the water circulation path from Gran Canaria toward La Palma and Gomera. Therefore, it is reasonable to hypothesize that populations from Gran Canaria might only succeed in interchanging migrants with Gomera, whereas individuals from Tenerife can easily accomplish dispersal toward the western islands of La Palma and Gomera. This hypothesis seems compatible with the sharing of exclusive alleles between Gran Canaria and Gomera, and with the IBD results. Moreover, this east–west direction of the genetic flux is a very common event in the models of colonization in the Canary Islands and shows a strong concordance between the topology of the cladograms and the distances of the islands from the continent which normally coincide with their geochronological sequence and usually explains the evolutionary and phylogeographic processes in these islands, in flora (Hess et al. 2000, Francisco-Ortega et al. 2000) as well as fauna (Thorpe et al. 1993, Juan et al. 1996, Marrero and Francisco-Ortega 2001).

These results do not concur with those described by Van der Strate et al. (2003) in the natural populations of *Cladophoropsis membranacea* in the Canary Islands. They did not detect IBD among the islands and interpreted their results in terms of non-equilibrium conditions, arguing that the structure of populations reflected some historical aspects rather than gene flow. On the contrary, our results conform very well with the IBD model. Therefore, the geological ages of the islands and the distances between them would explain the genetic flow model and, consequently the genetic structuring found in the populations of *Gelidium canariense* in the Canarian archipelago.

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