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Genetic structure of natural populations of *Gelidium* species: A re-evaluation of results

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Abstract

Twenty-two loci were re-evaluated to assess genetic variation and differentiation in three natural populations (two from Gran Canaria and one from Tenerife) of *Gelidium (G. canariensis* and *G. arbuscula*). The new data using exclusively the diploid subpopulation gene frequencies confirm that dispersal was restricted over short distances for the two species, but contrary to previous conclusions, the data reveal that these two closely related species differed markedly by their mating systems and patterns of genetic differentiation. Genetic differentiation among populations was twice as high in *G. arbuscula* as in *G. canariensis*. It was confirmed that the mean way of reproduction is asexual in *G. arbuscula* and a discussion included as to how clonal propagation may explain the difference in haploid and diploid allele frequencies in this species. There was no evidence for asexual reproduction in *G. canariensis*. Heterozygote deficiency could be explained simply by spatial sub-structuring within populations. The importance of the sampling design in determining the level and pattern of genetic differentiation within a species is discussed.

Introduction

Most studies that have investigated genetic structure in haplo-diploid algae (Fujio et al., 1985; Lindstrom, 1993; Sosa & Garcia-Reina, 1992, 1993; Williams & Di Fiori, 1996) have attributed the co-occurrence of low levels of genetic diversity and high levels of genetic differentiation to selfing and/or asexual reproduction. The only exception is *Halidrys dioica*, reported by Lu & Williams (1994); this is diploid and obligately outcrossing.

Most of the species considered are heteromorphic with an alternation of macroscopic and microscopic phases, though only the macroscopic phases were sampled. *Gelidium*, in contrast, is isomorphic so that both haploid and diploid phases could be sampled. Sosa and Garcia-Reina (1992, 1993) analysed and compared separately the genetic variation of haploid (female gametophytes) and diploid (tetrasporophytes) life stages in *G. canariensis* and *G. arbuscula* through isozyme electrophoresis. The authors calculated the Hardy-Weinberg departure and F-Statistics using the gene frequencies obtained from the haploid phases. The number of genes analysed for the haploid subpopulation was four times less than for the diploid one, leading to a possible bias. Therefore it was decided to re-evaluate the data using only the diploid subpopulation gene frequencies. Moreover, several procedures have been recently proposed in the literature to test for population structure (see Rousset & Raymond, 1997) which give additional and complementary information about the population structure of natural populations.

Table 1. Average genetic variation detected in *Gelidium arbuscula* and *G. canariensis* from natural populations of the Canary Islands. NG, Average Number of genes. NP, Number of analysed populations. NL, Number of loci. NA: Average Number of alleles. NPL: Average Number of polymorphic loci. A: Average number alleles per locus. P, Average Polymorphism. H, Average Expected heterozygosity. S, Sporophytic subpopulations. G, Gametophytic subpopulations. Data from Sosa and Garcia-Reina (1992, 1993).

		NP	NG	NL	NPL	NA	A	P	H
G. arbuscula	S	3	80	22	7	27	1.24	21.2	0.070
	G	3	15	22	2	23	1.06	6.0	0.018
G.canariensis	G	3	74	22	5	26	1.18	18.2	0.050
	G	3	15	22	3	22	1.10	9.1	0.031

Table 2. Tests for population differentiation among sporophytes and gametophytes within *Gelidium canariensis*. ns, not significant; –, no date; * $p < 0.05$; *** $p < 0.001$.

Populations	ALP-1	DIA-3	EST-2	MDH-1	PGI-3
Gáldar	–	ns	*	–	***
Agaete	ns	ns	ns	ns	ns
Puerto Cruz	–	ns	ns	ns	ns

The aim of this paper was to compare the two *Gelidium* species using these new tools for statistical analyses of population genetic data using diploid data. As no comparison of these two *Gelidium* species using isozyme variation has been published previously, their taxonomic status of was re-examined using Nei's genetic distance (Nei, 1978) for the isozyme data.

Material and methods

Species studied and sampling localities

Analysis of the genetic diversity of three natural populations of the Canary Islands, Galdar and Agaete from Gran Canaria, and Puerto de la Cruz from Tenerife of two *Gelidium* species (*G. arbuscula* and *G. canariensis*) was carried out by Sosa and Garcia-Reina (1992, 1993) using isozyme variation. The allelic frequencies for sporophytic and gametophytic phases were obtained separately. Twenty-three to 29 alleles were detected for *G. arbuscula*, and 23 to 27 alleles for *G. canariensis*, corresponding to twenty-two loci for both species (Table 1).

Statistical analyses

Deviation from Hardy-Weinberg expectations, genotypic linkage disequilibrium and differentiation among tetrasporophytic and gametophytic sub-populations were tested using the package GENEPOP, version 3.1 (Raymond & Rousset, 1995). For the two species, the test of HardyWeinberg proportions is an exact test, performed on the diploid sub-population data set, for each locus within each population. For both species, a Markov chain method was used to test for genotype linkage disequilibrium for each locus pair within each population using the diploid sub-population data set. Exact test for population differentiation among sporophytes and gametophytes within each species analyses the differences between haploid and diploid gene pools. For both species, exact tests for population differentiation were performed for each locus and within each population, also using a Markov chain method. In all cases, the Markov chain was set to 50,000 and 1000 steps of dememorization. The standard error was always <0.005.

The distribution of genetic variation within and among the natural populations of *Gelidium* species was analysed using F-statistics (Wright, 1965). F-statistics were computed according to Weir and Cockerham (1984) using the software FSTAT, version 1.2 (Goudet, 1995) on the diploid sub-population data set. GDA (Genetic Data Analysis: Lewis & Zaykin, 1997) software was used to calculate Nei's genetic identity (Nei, 1978). Nei's genetic identity and genetic distances (Nei, 1978) pair comparisons were calculated among populations, using diploid data set, within and among species to obtain the genetic relationship between both species.

Table 3. Tests for population differentiation among sporophytes and gametophytes within *Gelidium arbuscula*. ns, not significant; – no data; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Populations	ALP-1	DIA-2	EST-2	GDH-1	MDH-1	MDH-2	PGI- 3
Gáldar	–	***	***	ns	–	–	–
Agaete	ns	ns	**	ns	ns	ns	–
Puerto Cruz	–	ns	ns	–	*	ns	ns

Table 4. Tests for Hardy-Weinberg deviation for *Gelidium canariensis*. –: Not possible (monomorphic locus); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Populations	ALP-1	DIA-3	EST-2	MDH-1	PGI-3	Mean
Gálda	–	−0.28	−0.02	–	1.00***	0.49
Agaete	−0.14	0.37	0.03	0.00	−0.08	0.12
Puerto Cruz	–	0.09	0.02	−0.10	0.51**	0.20
Mean	−0.14	0.07	0.03	−0.09	0.65***	0.23***

Results

Exact test for sub-population differentiation among diploids and haploids gene frequencies

In *Gelidium canariensis* significant differences between the two phases were found only in the Gáldar population (Table 2). This population showed significant heterogeneity among haploid and diploid gene frequencies for two of the three loci studied: although the test showed only marginal significance for locus EST-2, the result was highly significant for locus PGI-3 (Table 2). Conversely, in *G. arbuscula*, a significant heterogeneity among haploid and diploid sub-populations was found within each population for at least one locus (MDH-1 in Puerto de la Cruz, DIA-2 and EST-2 in Gáldar, and EST-2 in Agaete: Table 3). Moreover, three of these comparisons were highly significant (Table 3).

Hardy-Weinberg tests

In *Gelidium canariensis*, only one locus (PGI-3: Table 4) showed significant deviations from the Hardy-Weinberg equilibrium (heterozygote deficiency). In *G. arbuscula*, four loci (ALP-1, EST-2, MDH-1, GDH-1: Table 5) showed significant deviations from Hardy-Weinberg expectations. For this species, heterozygote excesses were revealed for two loci (EST-2 and MDH-1) within each of the population studied. Heterozygote deficiencies were observed at the ALP-1 and GDH-1 loci within two of the three populations studied (Table 5).

Linkage disequilibrium

In *Gelidium canariensis*, only one test was marginally significant (MDH-1 and DIA-3 locus pair, data not shown) within Puerto de la Cruz population. In *G. arbuscula*, significant linkage disequilibria were detected within two of the three populations studied (Gáldar and Agaete for GDH-1/DIA-2 and MDH-2/DIA-2 locus pairs respectively (data not shown).

F-statistics

The significant heterozygote deficiency observed within *G. canariensis* species ($F_{IT} = 0.33$) was partially due to the within population component ($F_{IS} = 0.23$ essentially explained by locus PGI-3) and to the occurrence of differentiation among populations ($F_{ST} = 0.13$), (Table 6). In *Gelidium arbuscula*, the partitioning of total genetic variation observed within the species (Table 7) is very different than for *G. canariensis*. F_{IT} was essentially explained by a significant genetic differentiation among populations with a mean F_{ST} value of 0.28 being two times higher than in *G. canariensis*. The mean within population statistics was not significant because as we shown before, there was a strong discrepancy among loci and the ef-

Table 5. Tests for Hardy-Weinberg deviation in *Gelidium arbuscula*. –impossible (monomorphic locus); * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Populations	ALP-1	DIA-2	EST-2	GDH-1	MDH-1	MDH-2	PGI-3	Mean
Gáldar	–	0.26	−0.46**	0.17*	–	–	–	−0.06
Agaete	0.64***	−0.10	−0.54***	0.81***	0.25	−0.01	–	0.11
Puerto Cruz	–	−0.22	−0.26	–	−0.43*	−0.22	−0.06	−0.26
Mean	0.64***	−0.04	−0.45***	0.39***	−0.06	−0.19	−0.06	−0.06

fects of heterozygote deficits were counterbalanced by heterozygote excesses (Table 7).

Genetic identity

Table 8 shows the Nei's genetic identity, I, and the genetic distance, D, between all the populations of both *Gelidium* species. The average genetic identity between both species of *Gelidium* was 0.848, with values ranging from 0.824 to 0.876. These values were always less than 0.880 (Table 8). In contrast, the I values obtained between populations of the same species were always higher than 0.960. As shown with the F-statistics analysis, genetic identities among populations were lower for *G. arbuscula* than for *G. canariensis*. Thus, I within *G. canariensis* populations ranged from 0.986 to 0.998 (average = 0.992) and the I range within *G. arbuscula* was 0.960 to 0.978 (average = 0.972).

Table 6. F-statistics within *Gelidium canariensis*. *** $p<0.001$.

Locus	F_{IS}	F_{ST}	F_{IT}
ALP-1	−0.01	0.01	0.00
DIA-3	0.07	−0.01	0.06
EST-2	0.03	0.29	0.30
MDH-1	−0.09	0.07	−0.02
PGI-3	0.65	0.09	0.68
All loci	0.23***	0.13***	0.33***

Table 7. F-statistics within *Gelidium arbuscula*. *** $p < 0.001$.

Locus	F_{IS}	F_{ST}	F_{IT}
ALP-1	0.64	0.07	0.67
DIA-2	−0.04	0.50	0.48
EST-2	−0.45	0.08	−0.34
GDH-1	0.40	0.30	0.58
MDH-1	−0.06	0.16	0.11
MDH-2	−0.19	0.16	0.00
PGI-3	−0.06	0.16	0.00
All loci	−0.06	0.28***	0.24***

Discussion

Mating systems and within population structure

In a species with an alternation of diploid and haploid generations, sexual reproduction balances allele frequencies among the haploid and diploid subpopulations so that no genetic differentiation among the two phases should be expected (Coyer et al., 1994; Wattier et al., 1997). Conversely, asexual reproduction (haploids producing directly haploids and *vice versa* for diploids) should lead to a reduction of gene flow between the two generations. While asexual reproduction would tend to favour genetic differentiation *via* genetic drift (the effective size of an asexual population being reduced in comparison with a sexual reproducing one), sexual reproduction would tend to homogenise allele frequencies among haploid and diploid sub-populations. It might then be expected that genetic differentiation could occur between sporophytic and gametophytic subpopulations only if recruitment via sexual reproduction is lower than genetic drift. Thus, significant differentiation among diploid and haploid gene frequencies could be a good indicator of the extent of asexual reproduction in the two *Gelidium* species studied.

For *Gelidium arbuscula*, differences in allele frequencies were detected among sporophytes and gametophytes subpopulations in all of the three populations studied. These results confirm those described previously (Sosa & Garcia-Reina, 1992). Asexual reproduction was considered as an important way of

Table 8. Genetic distance (below) and genetic identity (above) (Nei, 1978) detected between sporophytic subpopulations of *Gelidium arbuscula* and *G. canariensis* from the Canary Islands. Average genetic identity within *G. canariensis* populations: 0.992. Average genetic identity within *G. arbuscula* populations: 0.972. Average genetic identity between *G. arbuscula* and *G. canariensis* populations: 0.848.

		Gelidium canariensis			*Gelidium arbuscula*		
	Population	Gáldar	Agaete	Pto. Cruz	Gáldar	Agaete	Pto. Cruz
G. canariensis	Gáldar		0.986	0.998	0.838	0.856	0.824
	Agaete	0.014		0.993	0.851	0.876	0.850
	Puerto Cruz	0.002	0.007		0.842	0.864	0.837
G. arbuscula	Gálddar	0.176	0.161	0.171		0.978	0.960
	Agaete	0.155	0.132	0.145	0.021		0.978
	Puerto Cruz	0.193	0.163	0.177	0.041	0.022	

propagation within *G. arbuscula* populations from the Canary Islands. Although we did not have experimental data about the importance of the sexual and asexual reproduction for *Gelidium arbuscula*, the introduction of new genotypes through sexual recruitment was apparently rare in most species of *Gelidium*. As discussed by Sosa and Garcia-Reina (1992) stoloniferous outgrowths of creeping axes was a common way of propagation of *Gelidium* species. In addition, the populations of *G. arbuscula* were stable in time (personal observation); therefore, locally adapted clones could propagate asexually and became predominant through competitive advantage, thereby minimizing the availability of unoccupied substratum and limiting opportunities for recruitment of new genotypes (via sexual reproduction). In this species, the detection of heterozygote excesses within all the populations studied in conjunction with linkage disequilibrium (observed for two of the three populations analyzed) confirmed clonal reproduction of this species. Even if inbreeding or spatial sub-structuring might be occurring, these results strongly suggested that *G. arbuscula* was mainly characterized by a high level of clonal reproduction.

In contrast, the significant differences in allele frequencies among sporophytic and gametophytic subpopulations for *G. canariensis* species were only observed for the Gáldar population. In addition, only one locus (PGI-3) showed a significant deviation from Hardy-Weinberg equilibrium, and the deviation was always towards a heterozygote deficiency. These results contradict those obtained by Sosa and Garcia-Reina (1992). Even if clonal propagation via fragmentation could not be totally rejected from these analyses, the results suggested that other reproductive strategies were influencing the within population genetic structure of this species. The significant differences in allele frequencies among sporophytic and gametophytic subpopulations for *G. canariensis* observed for Gáldar population could be explained by the occurrence of spatial sub- structuring (or Wahlund effect). Mixing of populations that differ in allele frequency would result in significant heterozygote deficiencies in the pooled or total population. In fact, the individuals analyzed for the Gáldar population belonged to five distinct successive samplings performed over two years within an area of less than one hundred square meters. Thus, for this population, the results could be explained by either a significant spatial or a temporal differentiation occurring within the site Gáldar. The observed heterozygote deficiency could be explained by selfing or inbreeding (sib-mating). However, the fact that heterozygote deficiency was only observed for a single locus suggested that this argument was relatively weak. A hierarchical and stratified sampling strategy would help to determine more accurately the spatial scale factors influencing the within population structure and to discriminate between the within-sub-population heterozygote deficiencies due to the breeding behaviour of the species (e.g. inbreeding) and the among sub-populations deficits due to the Wahlund effect (see Goudet et al., 1994 for more details). Finally, we could not exclude the hypothesis that selection (*via* hitch-hiking) was occurring at the PGI-3 locus to explain the discrepancy of the results among loci.

Genetic differentiation among populations within the species

Significant genetic differentiation among populations separated by 30 to 100 km was demonstrated in the two species. FST values ranged from 0.13 in *Gelidium* canariensis to 0.28 in *Gelidium arbuscula* indicating

low level of gene flow between populations. These values are higher than those described previously for diploid subpopulations (G_{st} was 0.073 and 0.147, respectively, for *G. canariensis* and *G. arbuscula*: Sosa & Garcia-Reina 1992, 1993), and revealed that long distance dispersal seemed to be insufficient to prevent genetic differentiation among populations. Consequently, we would expect that *Gelidium arbuscula* with vegetative reproduction would exhibit a higher level of differentiation among populations than *Gelidium canariensis* because genetic drift is more efficient in an asexual species characterised by a reduced effective population size. Data confirmed this prediction since FST was two times greater in *G. arbuscula* than in *G. canariensis*. Spatial genetic differentiation among algal populations have been recently discussed by Lu & Williams (1994) and Benzie et al. (1997). Even if little is known on population biology of seaweed, they suggested that as in terrestrial plants, most populations are structured geographically at different scales, resulting from a variety of factors including mating system and dispersal capabilities. In this paper, the re-evaluation of data obtained on the mating system of both *Gelidium* species gave a new insight for the observed patterns of population spatial structure.

Genetic distances between Gelidium species

Species boundary was confirmed by allozyme data. The genetic identities detected among populations of the same species were always greater than those between species. As discussed by Sosa & Garcia-Reina (1992, 1993), the conspecific genetic identity values for *Gelidium* populations were high, and comparable to those reported for other seaweeds and some land plants. At the same time, congeneric values detected for *Gelidium* species (I = 0.848) were also in the range described for other boundaries species (Cheney & Babbel, 1978; Benzie et al., 1997).

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