Genetic Diversity of the Macaronesian Leafy Liverwort *Porella canariensis* Inferred From RAPD Markers

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Plant colonization of the North Atlantic raises the intriguing question of the relationships between extant island species with their continental counterparts (European, African, and American), which may provide clues to past geographic distribution and colonization history. It has been suggested that during past glaciations, many plant species with typical Mediterranean distributions survived in the Atlantic islands that belong to what is today known as Macronesia. We used random amplified polymorphic DNA (RAPD) markers to study 12 populations of the liverwort *Porella canariensis* partly covering its present-day distribution (Azores, Madeira, Canary and Cape Verde Islands, and Iberian Peninsula). Unweighted pair-group (UPGMA) and principal component (PCO) analyses showed a similar geographical pattern that suggested a close relationship between Iberian populations and those from the Canaries and Cape Verde Islands. Populations from Madeira had more genetic variation than those from the Azores, a result from either a richer diversity of habitats in Madeira, which prompted more population diversification, successive colonization waves from different origins, or an older colonization of Madeira. The data show that continuous patches of liverworts are often comprised of more than one individual. Finally, RAPDs can be used to investigate intraspecific diversity within a comparatively large geographic area and, with utmost care, can be used to infer a historic context to explain the patterns observed.

In plant species, the amount of gene flow is a major factor determining genetic variation found throughout the species' distribution. Gene flow depends on such factors as reproductive mode, breeding system, and selective constraints (Loveless and Hamrick 1984; Mishler 1988). In diploid, outcrossing species it is common that genetic variation among populations is less than that within populations (Loveless and Hamrick 1984). On the other hand, species that are primarily selfed, but short-lived, have most of their genetic diversity among populations (Hamrick and Godt 1996). Species with limited dispersal or that reproduce by selfing present a pattern of geographic genetic variation where drift may play the primary role. Such could be the case of the Macaronesian liverwort *Porella canariensis*.

The Macronesia region consists of the Atlantic islands of the Azores, Madeira, Canary and Cape Verde. It harbors an extremely rich endemic and relict flora which in some places fully characterizes the main elements of the Tertiary European flora (Laurasia). Madeira harbors an extremely well-preserved Tertiary forest called Laurisilva which also exists in a much smaller extension in the Canary Islands. In the Azores, Cape Verde Islands, and Iberia, few elements of this typical forest are present. There is some evidence, however, that Laurisilva also existed on the coast of Morocco (Aubreville 1976). The intermediate position of these archipelagos between three continents makes them extremely interesting for studies of relict taxa of Gondwanalandic or Laurasian origin. Many of these taxa belong to the Macaronesian bryophyte flora, which includes more than 600 species, some of them with worldwide enigmatic distributions. Of these, 9% are exclusively endemic, some with direct relationships to other regions of bryophyte endemics as far away as Australia (Sérgio 1984).

The leafy liverwort genus *Porella* comprises more than 100 species with a worldwide distribution. Most of the taxa are found in southwestern Asia, where the genus is believed to have originated (Hattori 1978). In Europe, only eight species are currently recognized. The genus is known because of its wide phenotypic plasticity and morphologic variation which renders

ble 1. Location and sample numbers of *P. canariensis*

ample code e)	Location	Region	Elevation (m)/plant associations[a]	Poly-morphic sites (%)	Genetic diversity[b]	Genetic diversity[c]
(20)	Pico	Azores	500 c	71.2	2.9	.535
(20)	Faia1	Azores	720 a	76.2	3.1	.513
1 (20)	Machico	Madeira	320 d	68.7	2.8	.521
2 (20)	Ribeiro Frio	Madeira	800 a	62.5	2.5	.557
3 (14)	Porto Santo	Madeira	600 c	35.0	2.0	.784
4 (20)	Deserta	Madeira	450 d	71.2	2.9	.524
5 (20)	São Vicente	Madeira	900 a	73.7	3.0	.506
(20)	Sintra	Portugal	400 c	41.2	1.6	.711
(20)	Sintra	Portugal	400 c	72.5	2.9	.546
(20)	Tenerife	Canary	700 b	46.2	1.9	.702
2 (20)	Tenerife	Canary	900 a	27.5	1.2	.832
(10)	São Vicente	Cape Verde	760 d	46.2	3.7	.692

[a] ...aurisilva, humid; b, Laurisilva, dry and exposed; c, mixed forest, humid; d, coastal vegetation.

[b] ...io of the number of polymorphic RAPD markers within a population over the total number of individuals ...ampled.

[c] ...e diversity as $D = 1 - \Sigma fl_i^2$ where l_i is the frequency of the ith allele at the l locus (Weir 1996), which is ...ntical to H_e for random mating populations.

...ssification based on morphology prob-...matic (Hattori 1978). Molecular tech-...ques have proved invaluable for detect-... genetic differences among taxa ...oisselier-Dubayle and Bischler 1989, ...94; Therrien et al. 1998). *P. canariensis* ... an endemic species present on all Ma-...onesian islands. Eggers (1982) lists it for ...ven Azorean and five Canarian islands, ...t not Cape Verde and Deserta Island in ...adeira. On the Iberian Peninsula, the ...ecies is found in Sintra (Portugal) and ...geciras (south of Spain), but Jelenc ...55, in Ros et al. 1999) lists it for North ...rica, a distribution that suggests a dual ...gin for this species: Mediterranean and ...rican. *P. canariensis* occurs in small iso-...ed colonies or patches, depending on ... availability of specific ecological re-...irements such as atmospheric humidity ...d shade. Patches grow horizontally on ...es or rocks to 10–50 cm in diameter and ... found from sea level to 1700 m alti-...le.

...The founding and genetic composition ...these colonies is a matter of discussion ...ischler and Boisselier-Dubayle 1997 and ...erences herein). They may start with ...e or several individuals reproducing ...getatively or sexually. It is accepted that ...ese colonies are apparently reproduc-...ely isolated (Dewey 1989; Szweykowski ...82; Yamazaki 1982; Zehr 1980). Like ...ost liverworts, *P. canariensis* has a life ...cle characterized by the presence of a ...minant haploid gametophyte and a ...ort-lived diploid sporophyte. Spore dis-...rsal is usually limited to short distances ...nsson and Söderström 1988; Levin ...79) and sperm dispersal by water does ...t go far (from 4 to 65 cm) (Bischler and ...isselier-Dubayle 1997; Wyatt 1985). It is

widely accepted that liverworts have lim-ited dispersal abilities, in part because they easily reproduce vegetatively and most of their life cycle is haploid. Vegeta-tive propagation is perhaps more common than sexual reproduction, and isolation of populations should be very effective, even within short distances. Transport of prop-agules requires the existence of nearby streams, which are not always present. On the other hand, it has been shown that even low rates of sexual recruitment are sufficient to maintain some genetic diver-sity (Watkinson and Powell 1993). Also, al-lozyme studies have shown that clonal plants present a remarkable genetic vari-ation, even with low levels of sexual re-cruitment (Ellstrand and Rouse 1987; Wid-én et al. 1994).

If propagation of *P. canariensis* is mainly vegetative, drift could be the main force determining intraspecific differentiation, and high levels of homogeneity would be expected within populations. Self-fertiliza-tion within isolated colonies, especially if colonies are founded by a few individuals, tends to decrease clonal diversity. There is no strong evidence that selection plays a substantial role in determining any ge-netic variation among colonies of *P. can-ariensis* (Bischler and Boisselier-Dubayle 1997). Traditional methods of genetic analysis (isozyme or morphology) applied to these liverworts often fail to reveal var-iation in most conspecific populations, which implies restricted gene flow (Bois-selier-Dubayle et al. 1995; but see a con-trary view for other bryophytes in Stone-burner et al. 1991). This is consistent with a pattern of dispersal and founder events (Shaw 1991; Shaw and Schneider 1995). As an alternative, Wyatt et al. (1989b) pro-

posed that intrapopulation differentiation could be due to selection over different genotypes acting because of environmen-tal heterogeneity. Boisselier-Dubayle and Bischler (1994), working with random am-plified polymorphic DNA (RAPD) markers, reported the existence of some intraspe-cific genetic variation in several species of European *Porella*.

To bring new insights to the debate over the genetic composition and breeding sys-tems of *P. canariensis* populations, we used RAPD (Williams et al. 1990) banding patterns as markers to analyze intra- and interspecific genetic variation. These markers, presumed to be selectively neu-tral, should indicate that any pattern of variation found between different popula-tions would be caused by historical ef-fects, drift, and/or migration. This way, variation within populations must be de-termined by the mating system of the liv-erwort. The genetic relationships among the populations studied, partly covering the species distribution range, will be ad-dressed which may offer insights into the dispersal and colonization history of *P. canariensis* in Macaronesia.

Materials and Methods

Plant Material

Any genetic analysis of liverworts like *Po-rella* requires a clear definition of "popu-lation." We follow Bischler and Boisselier-Dubayle (1997) and define it as a continuous colony or "carpet" of liver-worts.

Ten populations of *P. canariensis* each represented by a patch 20–30 cm in di-ameter and belonging to the four Macro-nesian archipelagos (Azores, Madeira, Ca-nary Islands, and Cape Verde) and two from Portugal (Sintra) were used in this study (Table 1, Figure 1). The number of individuals ("shoots" or "ramets") from each population were randomly taken from each patch and are given in Table 1. Locations of the sampling sites are shown in Figure 1.

DNA Extraction

Before DNA extraction took place, all spec-imens were carefully washed and checked under the microscope to be sure that no possible contaminants (microalgae, fungi) were left. DNA extraction of specimens about 2–3 cm long and weighing just 10 mg required modification of several stan-dard protocols (Doyle and Doyle 1988; Murray and Thompson 1980; Rogers and Bendich 1985). We ground 10 mg of fresh
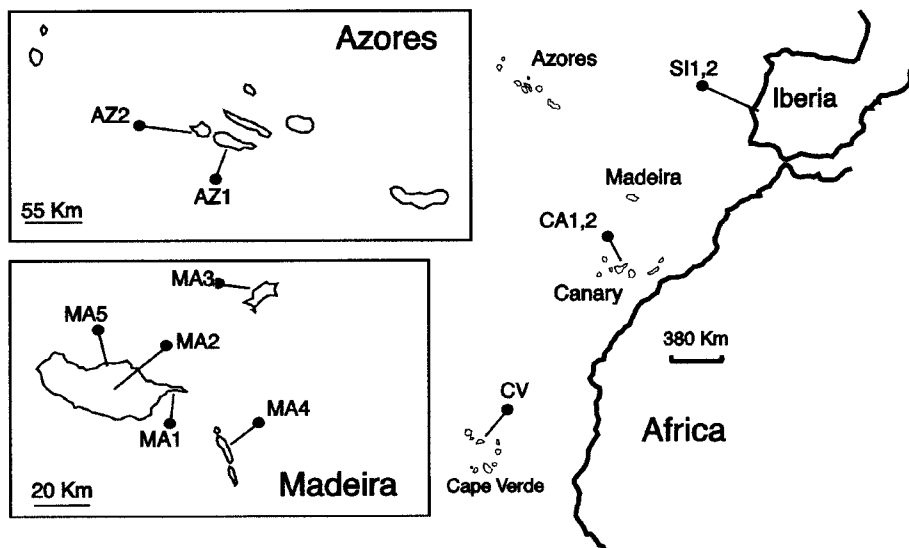
Figure 1. Geographic locations of the 12 populations of *P. canariensis* used in this study. Population codes are given in Table 1.

material (equivalent to one individual of *Porella*) to a fine powder in an Eppendorf tube with a glass rod and liquid nitrogen (tubes are kept with the tissues and filled with nitrogen for only a few s). The powder was then mixed with 500 ml CTAB buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 2% p/v CTAB; 20 mM EDTA4Na), 1% β-mercaptoethanol, 2% proteinase K (20 mg/ml), and then incubated in a bath at 65°C for 30 min. We added 335 ml of chloroform and 14 ml of 1-octanol to this mixture, followed by centrifuging 15 min at 13,000g. The supernatant was transferred to new Eppendorf tubes, mixed with 2/3 (v/v) isopropanol, and centrifuged for 15 min at 13,000g. Supernatant was discarded and the pellet washed with a solution of 76% ethanol AcNH$_4$ 10 mM for 20 min. This solution was centrifuged again for 10 min at 13,000g, the aqueous phase discarded, and the pellet resuspended in 100 ml of ultrapure water. Ten milliliters of AcNa 3 M (pH 5.2) and 250 ml of −20°C absolute ethanol were added, followed by a gentle mix and centrifugation for 15 min at 13,000g. The aqueous phase was removed and the pellet washed again with 70% ethanol and centrifuged as before. Finally, the aqueous phase was discarded and the pellet resuspended in 100 ml of ultrapure water or Tris-EDTA. This procedure recovered at least 0.5 mg of good-quality DNA for RAPD analysis.

Primers and PCR
Ten-mer primers from OPERON Technologies (Alameda, CA; series OPA-A,F,G,H,I,J) were screened on individual representatives of the populations under study.

Many of the primers produced either complex banding patterns or nonreproducible and inconsistent amplification products. Hence only 10 primers were chosen for the subsequent analysis. For each primer, only bands that could be unambiguously interpreted across all the population samples were chosen. Reproducibility of bands was assessed by replicating amplifications of samples selected at random. Polymerase chain reaction (PCR) was carried out in 20 μl volumes using 2 μl DNA (20 ng/μl), 2 μl dNTP mix (2 mM of each of four nucleotides; Promega), 2 μl 10× *Taq* DNA buffer (Pharmacia), 2 μl oligonucleotide primer (5 pmol/μl), 1 unit *Taq* DNA polymerase (Pharmacia), and the remainder of water. PCR conditions were 1 min at 92°C, 1 min at 36°C, and 2 min at 72°C, for 45 cycles, performed in a Biometra thermal cycler. Amplified products were separated in 1.2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet (UV) light. The 100 BPL (Pharmacia) was used as a molecular size marker.

Data Analysis
RAPD profiles were scored for each individual as discrete characters (presence or absence of amplified products) across all individuals from all populations and for each primer used. Table 1 shows chosen parameters for genetic diversity within populations: the percentage of polymorphic sites (markers), the genetic diversity calculated as the ratio of the number of polymorphic RAPD markers found in a population over the total number of individuals sampled, and Weir's D gene diver-

sity. The second parameter may be less influenced by the sample size, but Weir's D is a good indicator of variability especially for telling species (Weir 1996). An analysis of molecular variance (AMOVA) was performed based on Euclidean distances between all pairs of haplotypes according to Excoffier et al. (1992) and using the Arlequin program (Schneider et al. 1997). The total genetic variation could be divided into three levels of grouping using the five recognizable groups of populations: among regions (archipelagos), among populations within regions, and among individuals within populations. AMOVA components were tested for significance by nonparametric randomization tests using 6000 permutations under the null hypothesis of no population structure. A test for differences in intrapopulation molecular variances was also performed based on Bartlett's statistics (Excoffier et al. 1992; Stewart and Excoffier 1996). Additional AMOVAs were carried out to investigate the among-group variances allocated for each of the five groups of populations. In this case, only two groups were considered, the particular one being tested and the remaining populations combined. Based on nested variance components, the AMOVA gave Φ statistics, thus allowing the use of Φ_{ST} values (an estimate of F_{ST}; Excoffier et al. 1992) as interpopulation differentiation measures and the computation of $N_e m$ [$N_e m = 1/4(1/\Phi_{ST} - 1)$] as an estimator of gene flow (Slatkin and Barton 1989; Wright 1951). Significance of Φ_{ST} values were determined using a nonparametric permutation procedure. Variation among all pairs of samples was evaluated according to the index of genetic similarity of Nei and Li (1979), which is solely based on the shared presence of characters and excludes shared absence as a criterion of similarity. Relationships among populations were evaluated via the unweighted pair-group method (UPGMA) of Sneath and Sokal (1973) using Nei's (1978) genetic distance, and principal component (PCO) analysis. All analyses were performed using NTSYS (Rohlf 1993).

Correlation of pairwise Φ_{ST} values with geographic distances separating the populations (within and among sites) was assessed using a Mantel test. The significance of Mantel's Z statistics was calculated through a normalized approximation.

Results
The 10 primers amplified 80 RAPD bands in the 222 individuals of the 12 popula-

ble 2. Analysis of molecular variance (AMOVA) based on RAPD data for *P. canariensis*

rce of variation	df	SSD	MSD	Variance component	Total (%)	P^a	Φ_{ST}	Bartlett's index
ong archipelagos	4	1184.94	296.94	3.74	19.30	<.0002	0.603	(Bg) 4.21*
								(Bp) 11.73*
ong populations within archipelagos	7	1120.77	160.11	7.97	41.04	<.0002		
ong individuals within populations	210	1617.97	7.70	7.70	39.66	<.0002		

ted with nonparametric randomization tests (6000 permutations).

.0001.

= sum of squared deviation, MSD = mean squared deviation, Bg = variance heterogeneity among groups, Bp
ariance heterogeneity among populations.

ns studied. The number of polymorphic es varied considerably among the pop- tions studied (Table 1). Total genetic versity, estimated as the ratio of poly- orphic sites within a population to the mber of individuals sampled (Table 1), d the lowest value in the Canaries (1.2) d the highest in Cape Verde (3.7). Weir's 86) *D* values of gene diversity varied m 0.506 in MA5 to 0.832 in CA2, but the ues did not show any pattern of geo- aphic association. No relationship was und between genetic diversity with ei- r the type of associated vegetation NOVA, $F = 0.01$, ns) or altitude ($F = 1.4$,). Markers within populations were und to be randomly associated (data not own). The AMOVA results (Table 2) in- ate significant genetic differences be- een regions ($P < .0002$) and between pulations within regions ($P < .0002$). Of e total genetic divergence, only 19.3% s due to differences among the five re- ns (four archipelagos and the Iberian pulations). Most was attributed to dif- ences among populations within an ar- ipelago (41%) and variation within pop- tions (40%). Genetic variation within chipelagos was almost equally divided ong populations (58.6%) and within pulations (41.3%). Finally, more of the riation among archipelagos was due to ferences within (64.1%) rather then be- en (35.8%) them. Bartlett's heteroske- sticity indices showed that variance terogeneities differed significantly ($P <$ 01) among populations ($B_p = 11.73$) d among archipelagos ($B_g = 4.21$). Sep- te AMOVAs done with all groups

pooled except one (Table 3) showed that the Cape Verde archipelago contributed a lower percentage of variation over the to- tal (0.83), and Madeira contributed the highest (14.3). Analysis of variance done for each archipelago independently, showed that variance within populations was higher than variance among popula- tions in the Azores (68.06% and 31.94%, re- spectively) and Sintra (52.07 and 47.93), but not in Madeira (47.98 and 52.02) and the Canaries (32.17 and 67.83).

All Φ_{ST} pairwise values were significant ($P = .05$) and ranged from 0.32 between populations AZ1 and AZ2 to 0.82 between MA3 and CA2 (data not shown). Conse- quently the associated N_em values were al- ways less than 1, suggesting a low migra- tion rate between populations. The overall value of Φ_{ST} (0.603; Table 2) suggests sig- nificant heterogeneity for *Porella* popula- tions, indicating that the amount of differ- entiation is not due to heterogeneity among archipelagos.

UPGMA and PCO analyses revealed sim- ilar structuring of *P. canariensis* popula- tions, identifying the corresponding geo- graphical regions (Figures 2 and 3). Figure 2 includes two cluster analyses, one with Nei's (1978) genetic distance between the 12 populations and the other resulting from Nei and Li's (1979) similarity index between all 222 individuals. Because of the impracticability of presenting the lat- ter tree with so many individuals, we col- lapsed all individual branches from the same population to the nearest common node. This made it possible to compare both dendrograms. In both cases the five

populations from Madeira appear separat- ed from the others, in one clade. The ma- jor difference between both topologies is the position of the Azores and Cape Verde as outgroups for the closely related Ca- nary/Sintra populations. In the PCO anal- ysis (Figure 3), the first axis accounted for 21.3% of the total variance and clearly sep- arated the Madeira population, and to a lesser extent Cape Verde, from the others. This corresponds well to the first clade on the UPGMA analysis (Figure 2). The sec- ond axis accounted for 14.9% of the vari- ation and isolated both populations from the Azores. The third component (not shown) accounted for 12% of variation and separated population CA2 from the group Canary/Sintra. This component also discriminated population MA3 from the re- maining Madeira populations. Both these events are clearly depicted in the UPGMA clustering. The existence of three groups of populations—Madeira, Azores, and the others—is clearly distinguished by the PCO. Populations from Sintra were closer to the Canary ones, and Cape Verde oc- cupied an intermediate position between this last group and Madeira. Finally, pair- wise values between populations did not show any significant correlation with the geographic distance separating them, as revealed by a Mantel test ($r = 0.103$, t test $= 0.45$, ns).

Discussion

A substantial amount of variation in RAPD profiles of *P. canariensis* was found within populations (39.6%) and among popula- tions within each archipelago (41%). Much less variation (19%) was attributed to dif- ferences among regions (archipelagos), in spite of the fact that big geographic dis- tances separate them. Similar results were found by Martin et al. (1997) and Sale et al. (1996), but Liao and Hsiao (1998) and Hsiao and Lee (1999) found that variation among regions contributed more than var- iation among populations within regions to the total variation. Buso et al. (1998) found 77% of variation between regions versus 12% due to variation within re- gions. Gurgeli et al. (1999) reported 95% of variation within populations and only 1% among regions in the outcrossing and monoclonal *Saxifraga oppositifolia*. No congruence was found by these authors between genetic and geographic distanc- es. In our analysis of *P. canariensis*, varia- tion within populations in RAPD haplo- types (which made every ramet in the patches sampled a different genotype)

ble 3. Analysis of molecular variance (AMOVA) based on data from all regions pooled except one

rce of variation	Cape Verde	Madeira	Azores	Canary	Sintra
ong archipelagos	0.83	14.30	7.01	6.99	10.44
ong and within populations	99.17	85.70	92.99	93.01	89.56
	0.589	0.614	0.606	0.606	0.615

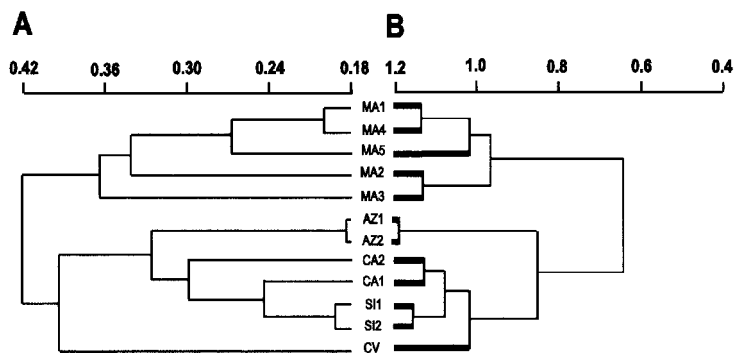values are significant after 10,000 permuations ($P < .0001$).

Figure 2. Cluster analyses (UPGMA) of 12 populations of *P. canariensis* based on 80 RAPD markers using Nei's (1978) genetic distance (A), and Nei and Li's (1979) similarity index (B). Thicker branches mean that individual branches in each population were collapsed into one. Population codes are as in Table 1.

contradicts the homogeneity found in morphology, even between populations that are localized in different environments. We have sequenced several individuals from different populations of *P. canariensis* for the ITS region of nuclear rDNA and found no polymorphism (unpublished results).

The high variation within populations revealed by the AMOVAs and the fact that no identical haplotypes existed within any colony indicates that the differences observed might correspond to different individuals sampled from the same patch. An alternative explanation could be that *Porella,* which reproduces vegetatively during most of its long haploid life cycle, accumulates mutations, producing an extremely rich clonal diversity. Individuals sampled in the adult haploid phase would make mutations easily detectable, explaining the high variability found. Waycott (1998), in a study of uniclonal populations of sea grasses, proposed that long-lived individuals characterized by vegetative

growth may have accumulated genotypic differences via the accumulation of somatic mutations and recombination, a fact also predicted in theoretical models by Soane and Watkinson (1979). Gabrielsen et al. (1998) also reported high levels of genetic diversity in the arctic clonal *Saxifraga cernua,* and Cronberg et al. (1997) explained the high genetic variation found in a clonal bryophyte as due to a perennial stayer life strategy associated with wide wind dispersal of pollen. In contrast, Bauert et al. (1998) found three isolated, relict alpine populations of the same species presented no variation at all. This was explained by postglacial events resulting in bottlenecks or founder events. Also Steinger et al. (1996) reported that patches of slow and clonally growing *Carex curvula* are genetically uniform. In spite of the fact that the breeding system of *P. canariensis* is poorly understood, it is known that the species reproduces well vegetatively (Sá-Fontinha S, personal observation) and that each individual can live and
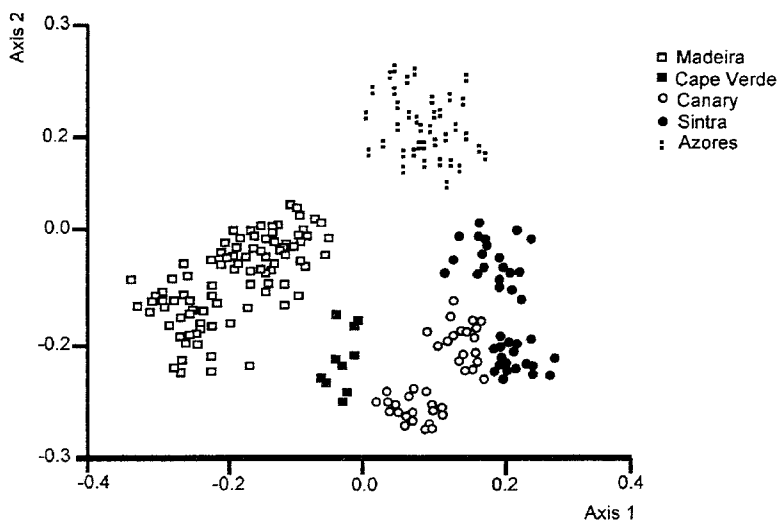
grow for a long time. In this case, recombination is possible among individuals of different generations during the short period of their diploid and sexual cycle, and vegetative growth preserves these acquired genotypes.

At least in the Madeira archipelago, mature plants in full reproductive phase have been observed in some populations (patches), but no evidence exists that sexual reproduction is the major factor contributing through recombination to the high level of genetic variability. Observations in other populations, like Porto Santo, only rarely show reproductively mature plants (Sá-Fontinha S, personal observation). The fact that RAPD markers were found to be randomly associated is evidence that some sexual reproduction exists within each patch. The widely accepted lack of substantial spore dispersal in *P. canariensis* is supported by 41% of the variation being attributed to differences between populations within archipelagos. Such a pattern suggests that *P. canariensis* is a typical outcrossing species according to Loveless and Hamrick (1984) and Hamrick and Godt (1996). In this case, however, high levels of variation still exist among populations within regions, especially in the case of Madeira, with five populations sampled, which indicates that some reduced gene flow exists. The fact that the species is not widespread, but rather is confined to particular habitat conditions where the colonies can grow, increases variation among populations belonging to the same group. The AMOVA also shows that differences among archipelagos are maximum between the Madeira populations and the others (14%) and minimum between the population of Cape Verde and the others (0.8%). These values may be inflated, however, because the samples from the Canaries are from the same island, and only two islands were screened for the Azores.

RAPD profiles revealed a structuring of populations completely congruent with their geographic location. Variation found within Madeira is high if we compare it to both populations from Tenerife and even to the two Azorean samples on different islands. Both cluster analyses and PCO showed similar grouping of populations and clearly separate the Madeira cluster from the others. In the Madeira group of populations, the two more closely related samples are MA1 on Madeira Island and MA4 on the Desertas Islands. Both populations appear in coastal vegetation which is not the case of MA2, which occurs in

Figure 3. PCO analyses of 222 individuals of *P. canariensis* from 12 populations. Axis 1 extracted 21.3% of the total variation and axis 2, 14.9%.

urisilva forest. In the other group of populations, the two trees slightly differ in the clustering of internal nodes. Nevertheless, both agree in placing together the populations from Sintra and Tenerife (Canaries). The populations from the Azores always cluster together and are the two most similar ones. Their place as an outgroup for the Sintra/Tenerife populations may change with Cape Verde according to the clustering method used. On a strict simple-match basis of similarity, it is clear that Cape Verde joins with both the Tenerife and Sintra populations, and only then joins the two Azores. This has implications for the general pattern of dispersion and colonization history of *P. canariensis*. Mediterranean in origin, the species may have occupied a much broader area than it does today. In fact, the data allow us to speculate that *P. canariensis* may have existed from Iberia to the coast of Cape Verde when the Sahara was green, much of its range then was probably associated with the former Tertiary Laurisia rain forest. This type of forest is today completely absent from Morocco, but some evidence suggests that it had previously existed (Aubreville 1976). In Cape Verde the last few colonies of *P. canariensis* are quickly disappearing with desertification. We have only found it on São Vicente Island on a mountain where very few Laurasian elements still exist. Starting from the mainland, colonization of the Cape Verde and Canary Islands would be due to the proximity to the coast, probably using other plant species as carriers.

The results from the AMOVA analyses, together with the lack of correlation between pairwise F_{ST} values and geographic distances among the 12 samples studied, indicate that habitat differentiation is more important than large-scale geographic differentiation (isolation by distance) to account for variation in these populations of *Porella*. High levels of genetic variation found in bryophytes by Wyatt et al. (1989a) have been explained as genetic adaptations to microhabitats. In fact, the variance among regions is smaller than that among populations within a single region. This result could also be due to the fact that more samples were used in Madeira than in the Canaries or the Azores, but similar results were obtained when we performed the analysis with just the three Madeira Island samples. Large distances between the archipelagos and Sintra (1400 km between Sintra and Canary, 2600 km between Sintra and Cape Verde, 1000 km from Sintra to either Madeira or

Azores), and the fact that many extant populations are rare and confined to specific areas, make present-day gene flow among regions virtually impossible. The relatedness of populations from Sintra, Canary, and Cape Verde as revealed in the dendrograms indicates that part of their RAPD markers are shared and date back to a single large population. Particular habitat conditions on Tenerife probably prevent the two populations analyzed from diverging further. Populations from the Azores may be considered a separate founding event from continental populations. The Azores are the youngest islands and have low habitat diversity. This could explain why the two populations belonging to different islands are separated by a small genetic distance.

Taking into consideration variation within populations of *P. canariensis*, it is clear that colonies are comprised of more than one individual (i.e., multiclonal). Moreover, besides vegetative reproduction, sexual recombination appears to be more common than is usually admitted. The high variation among populations within regions confirms that spore dispersal, even over short distances, is probably only effective within colonies. Ecological constraints may be a strong factor limiting the possibility for colonies to diverge. Finally, the good agreement obtained between the two dendrograms suggests a continuous, large population of *P. canariensis*, at least from Iberia to the coast of Cape Verde in mainland Africa, from which the plant colonized the two southern archipelagos. The close relationship between *P. canariensis* populations from these Atlantic archipelagos and the samples from Sintra corroborates the hypothesis that some of the *P. canariensis* polymorphism is not recent, but rather dates back to a preglacial widespread population that found refuge in these habitats that were essentially untouched by climatic changes.

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