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REDUCED GENETIC DIVERSITY AND INCREASED POPULATION DIFFERENTIATION IN PERIPHERAL AND OVERHARVESTED POPULATIONS OF *GIGARTINA SKOTTSBERGII* (RHODOPHYTA, GIGARTINALES) IN SOUTHERN CHILE[1]

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This study assesses two hypotheses on the genetic diversity of populations of *Gigartina skottsbergii* Setchell et Gardner (Rhodophyta, Gigartinales) at the border of the species distribution: 1) peripheral populations display a reduced genetic diversity compared with central populations, and 2) genetic differentiation is higher among peripheral than among central populations. Two peripheral and four central populations were sampled along the Chilean coast and 113 haploid individuals were analyzed using 17 random amplification of polymorphic DNA loci. The genetic diversity was estimated by allele diversity (H_e), allele richness ($\hat{A}$), and the mean pair-wise differences among multilocus genotypes. All three estimates consistently and significantly indicated a lower genetic diversity within the peripheral than within the central populations. Genetic differentiation between the two peripheral populations was stronger ($F_{ST} = 0.35$) than between central populations at similar spatial scales (F_{ST} ranging from 0 to 0.25). In addition, it appeared from the distribution of pair-wise differences that peripheral populations are in demographic expansion after a recent bottleneck. The results are discussed in the specific context of potential overharvesting of these wild populations.

Key index words: **genetic diversity; mismatch distribution; overharvest; RAPD; Rhodophyta; seaweed; species border**

It is now well established that the distribution of most species is characterized by central dense populations clustered together and peripheral marginal populations (Brown 1984, Brown et al. 1995). This pattern of distribution is regulated by biotic and/or abiotic factors that make peripheral habitats less suitable for the maintenance of populations (Brown et al. 1996). The exact range boundary is determined by the interaction of demographic characteristics of the marginal populations and dispersal rate with the spatial and temporal variability of the environment (Brown et al. 1996, Holt and Keitt 2000, Maurer and Taper 2002). At least two features are considered important in defining marginal populations. First, they are generally fragmented in patches that follow the patchy nature of the marginal habitat (Brown et al. 1996), a feature that favors metapopulational processes (Holt and Keitt 2000). Second, because marginal habitats are less suitable than central ones, individual fitness and population growth rates are expected to be reduced at the species range boundary (Holt and Keitt 2000, Pulliam 2000, Maurer and Taper 2002). Marginal populations should thus present a reduced effective size and experience strong genetic drift. As a consequence, there should be a tendency toward a reduced genetic variability within the peripheral populations compared with those at the center of the species distribution (Ledig 1986) and an increased genetic differentiation among them. However, genetic differentiation of the peripheral populations can also arise through local adaptation to marginal ecological conditions (van Rossum et al. 1997), overwhelming the possible effects of genetic drift and making these populations of particular interest for conservation purposes (Lessica and Allendorf 1995).

The reported studies on differences in genetic diversity between central and peripheral populations (Bouza et al. 1999, Durka 1999, Lammi et al. 1999, Jones et al. 2001, Pedersen and Loeschcke 2001, Lönn and Prentice 2002) tend to confirm two issues on genetic polymorphism. First, genetic diversity, estimated either as number of alleles, heterozygosity, or as sequence variation, is reduced in small and marginal populations compared with central and dense populations (but see van Rossum et al. 1997). Second, genetic differentiation is stronger among peripheral populations than among central populations.

Superimposed on the effect of marginal ecological conditions at the range boundary, human impact can also affect species border in different ways, leading to contrasting situations. For example, human activities

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can favor long distance dispersal across a biogeographic boundary, expanding the range of a species distribution by introducing individuals in new sites or habitats. Conversely, human activities often lead to a fragmentation or to a reduction of suitable habitats, which may result in a reduction of a species' range. Humans can also directly influence the abundance of a given species by overexploitation (i.e. overharvesting), causing a reduction in population size and a modification of population dynamics (Vitousek et al. 1997) and, ultimately, a loss of genetic diversity (Buchert et al. 1997). Such influence may dramatically reinforce local genetic erosion in peripheral populations.

For marine organisms, most information on this issue comes from fisheries, where overfishing often leads to population depletion (Ludwig et al. 1993, Policansky 1996, Golsworthy et al. 2000, Pauly et al. 2002) and community and ecosystem degradation (Botsford et al. 1997). Furthermore, the naturally reduced genetic diversity at the species border may suffer additional reduction when overexploitation occurs, as in the case of the New Zealand snapper (Hauser et al. 2002). These studies are, to our knowledge, absent for macroalgae, despite the clear evidence that their natural stocks are being increasingly affected by overharvesting (Billot et al. 2003).

In this study we used the red alga *Gigartina skottsbergii* Setchell et Gardner as a model to assess the validity of the generalizations indicated above. This subtidal species inhabits the archipelago region in southern Chile and Argentina, displaying a highly fragmented distribution along Antarctic and sub-Antarctic waters, with stands usually smaller than 20 km^2. The northern limit of its distribution (40°S) is set by the limit of the coldest water of South America and extends up to the Antarctic region (Ramirez and Santelices 1991). Marginal populations at the northern limit are characterized by relatively small size, low density (Piriz 1996 in Argentina; Avila et al. 1999 and Westermeier et al. 1999 in Chile), short reproductive period, and low recruitment rates (Zamorano and Westermeier 1996). This giant alga is perennial, and blades may reach up to 1–2 m in diameter (Santelices 1988). *Gigartina skottsbergii* only propagates through sexual reproduction, and both phases of the isomorphic life cycle coexist in time and space (Piriz 1996, Avila et al. 1999, Westermeier et al. 1999). In Chile, *G. skottsbergii* is one of the main sources of raw material for the carrageenan industry, and therefore it has become one of the most important algal resources (Buschmann et al. 2001, Avila et al. 2003). This species has been heavily harvested for more than a decade along its northern limit of distribution, leading to a severe reduction in population size and to a crash in the total landings (SERNAPESCA 2001, Avila et al. 2003). On the other hand, and despite recent information that suggests a harvesting effort moving southward (SERNAPESCA 2001), the effects of harvesting are still negligible in the southernmost regions (Avila et al. 2003).

The lack of information on genetic resources within *G. skottsbergii* populations, particularly in the context of overharvesting of wild populations (Avila et al. 2003), has led to concerns about conservation issues. This situation stresses the needs of gaining knowledge on the patterns and levels of genetic variation to establish the status of the exploited peripheral Chilean populations in comparison with central populations that are still not overharvested. This study tests the hypothesis that in *G. skottsbergii*, small, marginal, and overexploited populations along the northern limit of the distribution range display a reduced genetic diversity within populations and an increased among-population genetic differentiation. In contrast, larger central and southern populations are expected to present a higher level of within-population genetic diversity and lower among-population genetic differentiation. Genetic diversity was analyzed by random amplification of polymorphic DNA (RAPDs) on haploid individuals (gametophytes), which allows the direct detection of haploid genotypes and therefore avoids the problems of dominance generally observed when using diploid organisms (Lynch and Milligan 1994, Harris 1999, Sunnucks 2000).

MATERIALS AND METHODS

Studied populations and sampling. Six sites were chosen on the Pacific coast of southern Chile (Fig. 1, Table 1). These included two peripheral populations in the Xth region (Puerto Montt area), the northern limit of the species distribution that, in addition, concentrated most of the past harvesting efforts: Ancud and Calbuco. Four central populations were sampled in two regions. One population, Puerto Aguirre, was located in the XIth region, and the three other populations, Puerto Yartau, Bahía Inútil, and Bahía Chilota, were located near Punta Arenas, in the XIIth region. Distances separating the sampling sites ranged from 30 to 1150 km (Fig. 1).

Samples of *G. skottsbergii* were obtained by diving at depths of 9 to 15 m. Immediately after collection, immature and healthy pieces of 4 cm^2 were excised from the basal part of the fronds. They were rinsed with fresh water, blotted, and dried in silica gel. To identify haploid individuals, 1-cm^2 sections of fresh tissue were excised from each frond and brought to the laboratory to perform the acetal-resorcinol colorimetric test (Craigie and Leigh 1978). One hundred thirteen samples were determined as haploid (Table 1) and retained for genetic analysis.

DNA extraction and PCR conditions. Dry tissue was finely ground in liquid nitrogen, and 50 μL of the resulting powder was used for genomic DNA extraction following the protocol described by Saunders (1993). DNA yields were estimated by direct comparison with standard DNA concentrations in 1.0% agarose gels stained with ethidium bromide. The DNA was diluted in sterile deionized water to a final concentration of $10\ ng \cdot \mu L^{-1}$.

Amplifications were carried out in a 20-μL reaction mixture containing 2 μL diluted template-DNA (20 ng), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 μM of each dNTP, 1.25 Units of Taq Polymerase (GIBCO, Rockville, MD, USA), and 1.5 μM of primer (Operon Technologies, Alameda, CA, USA and GeneSet, San Diego, CA, USA). Amplifications were done in a GenAmp 9700 thermocycler (Perkin-Elmer, Boston, MA, USA) with an initial denaturation step of 94° C for 4 min

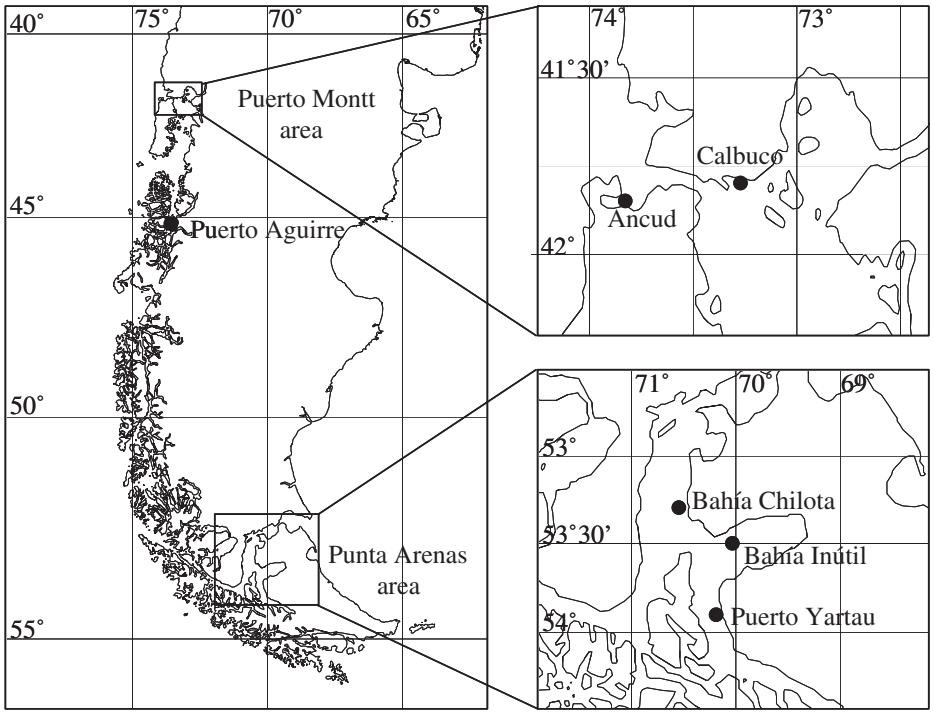

FIG. 1. Map of the sampled populations.

followed by 35 cycles of 1 min at 94° C, 1 min at 38° C, and 1.5 min at 72° C, and a final extension period of 5 min at 72° C. Amplification products were separated on 1.5% agarose gels in TBE buffer and visualized under UV light after ethidium bromide staining. A total of 80 primers from the Operon series A, B, D, and X were tested to select those producing reproducible and polymorphic profiles according to Harris (1999). Presence or absence of RAPD bands was scored from photographs for all tested primers. The two primers X8 (5′-GAGGGGTGGA-3′) and B1 (5′-GTTTCGCTCC-3′) were chosen for the unambiguous pattern of presence of thick and bright bands or absence of them (no band at all). Primers with tenuous bands or bands displaying inconsistencies in their exact position within the gel were excluded. Because only haploid individuals were analyzed, no genotypic information was lost due to the dominance of the RAPD marker (Harris 1999, Sunnucks 2000), and each band was considered as a locus with two alleles ("present" and "absent").

TABLE 1. Location of sampling sites for *Gigartina skottsbergii*, percentage of haploid individuals, and number of sampled individuals.

Population	Location	Number of sampled individuals	Percentage of haploids[a]
Calbuco	41°43′ S 73°05′ W	38	100
Ancud Bay	41°51′ S 73°49′ W	40	100
Puerto Aguirre	45°15′ S 73°30′ W	100	84
Bahía Chilota	53°18′ S 70°28′ W	26	85
Bahía Inútil	53°30′ S 70°07′ W	22	45
Puerto Yartau	53°53′ S 70°11′ W	22	95

[a]Percentage of haploids identified using the Resorcinol test (Craigie and Leigh 1978).

Genetic diversity within populations. To test whether different bands were independent or linked within the banding profile, gametic linkage disequilibrium (Lewontin and Kojima 1960) between RAPD loci was estimated using an extension of the Fisher's exact test of linkage disequilibrium (Slatkin 1994). Significance level of linkage disequilibria under the hypothesis of random segregation between allele pairs were determined by means of 10,000 permutations of alleles between individuals for each pair of loci, using the ARLEQUIN package (Schneider et al. 2000). Because multiple tests were conducted, a Bonferroni sequential procedure was used to correct the significance levels (Rice 1989). Allelic diversity was estimated as the unbiased estimate of heterozygosity (H_e) (Nei 1978) for each locus and population, using the GENETIX package, version 4.0 (Belkhir 1997). Allelic richness ($\hat{A}$), the mean number of alleles per locus per population (averaged over all loci), was calculated using the rarefaction procedure described in Petit et al. (1998) for unequal sample sizes (as recommended by Leberg 2002) with the FSTAT v2.9.3 software (Goudet 2001). Numbers of unique and shared multilocus genotypes and of pair-wise differences among genotypes within each population were counted using the ARLEQUIN package (Schneider et al. 2000).

To test for differences in genetic diversity between central and peripheral populations, two kinds of tests were performed. Each test compared the group of central populations, namely Puerto Aguirre, Puerto Yartau, Bahía Inútil, and Bahía Chilota, with the group of peripheral populations (Calbuco and Ancud). Mean H_e and mean pair-wise differences among genotypes were compared with a Mann-Whitney U-test, and $\hat{A}$ values were compared with a one-sided permutation test (H_1: $\hat{A}$ is greater in the central than in the peripheral group).

This latter test was performed with FSTAT software with 15,000 permutations.

Genetic differentiation among populations. Wright's F_{ST} index, estimator of genetic differentiation among populations (Weir and Cockerham 1984), and its associated probability were estimated for the whole set of samples and for all localities pair-wise comparisons. Significance levels of F_{ST} under the hypothesis of no differentiation between populations were determined by means of 10,000 random permutations of genotypes between samples. In this test, the P value is the proportion leading to an F_{ST} larger or equal to the observed value.

RESULTS

Primers X8 and B1 produced 12 and 5 bands, respectively, leading to a total of 17 loci with a clear and reproducible banding pattern. Frequency of allele "present" ranged from 0 to 1 depending on the locus and the population, but no one was monomorphic across the 113 samples (Table 2). In general, allele frequency was highly variable among populations, indicating a high overall genetic diversity and suggesting the occurrence of population differentiation. The southern central populations of Bahía Inútil and Puerto Yartau had the largest proportion of significant pair-wise linkage disequilibria (14.5%, Table 3), whereas the smallest proportion (8.3%, which is not negligible, Table 3) was found in the two northern peripheral populations (Calbuco and Ancud). The analysis did not show any systematic link between the same allele pairs across all populations, indicating that each locus could be considered as an independent variable. These observed linkage disequilibria might rather be explained by population substructure.

Genetic diversity within populations. Allelic diversity was moderate, with the maximum H_e value reaching 0.28 for Bahía Chilota and the lowest for the two peripheral populations (0.13 for Calbuco and 0.17 for Ancud) (Table 4). The differences between groups of central and peripheral populations were highly significant ($z = -2.60$, $P = 0.009$; Table 5), whereas differences among populations within each group were not significant (data not shown). Similarly, allelic richness was significantly higher in central than in peripheral populations ($P = 0.0095$, Table 5) with values ranging from 1.53 in Calbuco to 1.77 for Puerto Aguirre (Table 4). The number of different genotypes was always lower than the number of sampled individuals, and therefore some multilocus genotypes were observed in two or more individuals within each population. Calbuco and Ancud had about 8% of shared genotypes (Fig. 2), with up to eight individuals sharing the same genotype in Ancud, whereas central populations had only 0.5%–2% of shared genotypes (Table 4). In addition, the mean number of pair-wise differences among these genotypes was consistently lower for the two peripheral populations (2.10 for Calbuco and 2.92 for Ancud) and resulted in a Poisson shaped distribution of the frequencies of pair-wise differences among genotypes (Fig. 2). Conversely, it was always higher than 4.5 for the central populations (Table 4), and the frequency distribution of the pair-wise differences was skewed to right with several modes (Fig. 2). These differences were significant between the two groups of central and peripheral populations ($z = 17.02$, $P < 10^{-3}$; Table 5).

Genetic differentiation among populations. Pair-wise F_{ST} values were generally high and highly significant, except between Bahía Chilota and Bahía Inútil (Table 6). For geographic distances of 450 km or more, F_{ST} values ranging from 0.45 to 0.57 were observed. For shorter distances (from 30 to 70 km) F_{ST} values were smaller, ranging from 0 to 0.25, and were not related to the geographic distance (Table 6). Interestingly, the F_{ST} value between the two peripheral populations (Calbuco and Ancud) was higher (0.35) than F_{ST} between pairs of populations from the central area, indicating that these two peripheral populations are more genetically differentiated than the populations of the Punta. Arenas area.

TABLE 2. Frequency of allele "present" for each locus and each population.

	Calbuco ($n = 22$)	Ancud ($n = 31$)	Puerto Aguirre ($n = 19$)	Bahía Chilota ($n = 13$)	Bahía Inútil ($n = 10$)	Puerto Yartau ($n = 18$)
X8-1	0.50	1.00	0.16	0.46	0.40	0.78
X8-2	1.00	1.00	0.79	0.54	0.60	0.72
X8-3	1.00	1.00	1.00	0.54	0.80	0.33
X8-4	0.91	0.87	0.79	0.92	0.90	1.00
X8-5	0.00	0.32	1.00	0.31	0.40	0.50
X8-6	0.73	0.94	0.53	0.38	0.20	0.33
X8-7	1.00	0.81	0.95	0.62	0.60	0.56
X8-8	0.05	0.03	0.84	0.00	0.40	0.56
X8-9	1.00	1.00	1.00	0.62	0.80	0.33
X8-10	0.09	0.16	0.68	0.00	0.00	0.78
X8-11	1.00	1.00	0.63	0.00	0.00	0.00
X8-12	0.00	0.90	0.47	0.00	0.00	0.00
B1-1	0.91	1.00	1.00	0.92	1.00	1.00
B1-4	0.23	0.29	0.16	0.31	0.10	0.67
B1-7	0.95	0.45	0.68	0.00	0.00	0.00
B1-8	0.05	0.00	0.68	0.00	0.00	0.06
B1-9	1.00	0.81	1.00	0.54	0.30	0.94

TABLE 3. Number and proportion of significant pair-wise linkage disequilibria (after Bonferroni's correction for multiple comparisons) within each population.

Population	Number of comparisons	Number of significant pair-wise linkage disequilibria	Percentage of significant pair-wise linkage disequilibria
Calbuco	36	3	8.3
Ancud	45	4	8.8
Puerto Aguirre	78	7	9.0
Bahía Chilota	55	6	10.9
Bahía Inútil	55	8	14.5
Puerto Yartau	66	9	13.6

DISCUSSION

Comparison of central and peripheral population genetic structure. The present study confirmed the two main hypotheses concerning the genetic structure of peripheral populations of *G. skottsbergii*: 1) genetic diversity is reduced in peripheral and overharvested populations as compared with central and more pristine populations, and 2) genetic differentiation is higher among peripheral than among central populations. By comparing central and peripheral populations, it appeared that the genetic diversity, either as the allele diversity (H_e), the allelic richness ($\hat{A}$), or the genotype diversity, is significantly reduced in peripheral populations, validating our first hypothesis. This result is also in agreement with most of the published studies, although only a few tested the significance of these differences (Durka 1999, Lammi et al. 1999, Lönn and Prentice 2002). In contrast, van Rossum et al. (1997) did not find significant differences between central and peripheral populations of *Silene nutans*. These authors postulated that the maintenance of high levels of genetic diversity in small and marginal populations of this plant was due to a long life span, an outcrossing breeding system, and a long-distance dispersal capability. *Gigartina skottsbergii* is also an obligate outcrosser and considered as a perennial alga. However, short dispersal distances (reduced gene flow) and the additional effect of overharvesting (see below) seem to have increased local genetic drift in this species.

TABLE 4. Allelic and genotypic diversity.

	n	H_e	$\hat{A}$	N_g	MPD
Calbuco	22	0.128	1.529	13	2.10
Ancud	31	0.167	1.588	20	2.92
Puerto Aguirre	19	0.261	1.765	18	4.56
Bahía Chilota	13	0.278	1.647	12	4.92
Bahía Inútil	10	0.256	1.647	9	4.60
Puerto Yartau	18	0.276	1.706	16	4.84

Overlocus mean allelic diversity (as the unbiased estimate of expected heterozygosity, H_e; Nei 1978) and allelic richness, $\hat{A}$ (Petit et al. 1998), for each population, and mean number of pair-wise differences among genotypes (MPD) within each population. N_g, number of different multilocus genotypes.

TABLE 5. Results of the tests comparing mean expected heterozygosity (H_e), allelic richness ($\hat{A}$), and mean pair-wise differences (MPD) between the group of marginal populations and the group of central populations.

	Mean for central	Mean for marginal	Test	Results
H_e[a]	0.14 ± 0.03	0.27 ± 0.03	Mann-Whitney	$z = -2.598$ $P = 0.009$
$\hat{A}$	1.50	1.67	Permutation test	$P = 0.0095$
MPD[a]	2.64 ± 0.06	4.72 ± 0.09	Mann-Whitney	$z = -17.017$ $P < 0.001$

[a]Values are mean ± SE.

Indeed, our results indicated that peripheral populations are more differentiated than central populations (F_{ST} value is 1.5 to 2 times higher between marginal than between central populations for a similar geographic scale), validating our second hypothesis (although only two marginal populations were sampled, potentially limiting the generalization of the results).

Genetic isolation is expected for any population of *G. skottsbergii* when considering its normal dispersal capacity. Seaweeds are generally considered poor dispersers because spore survival is generally limited to a

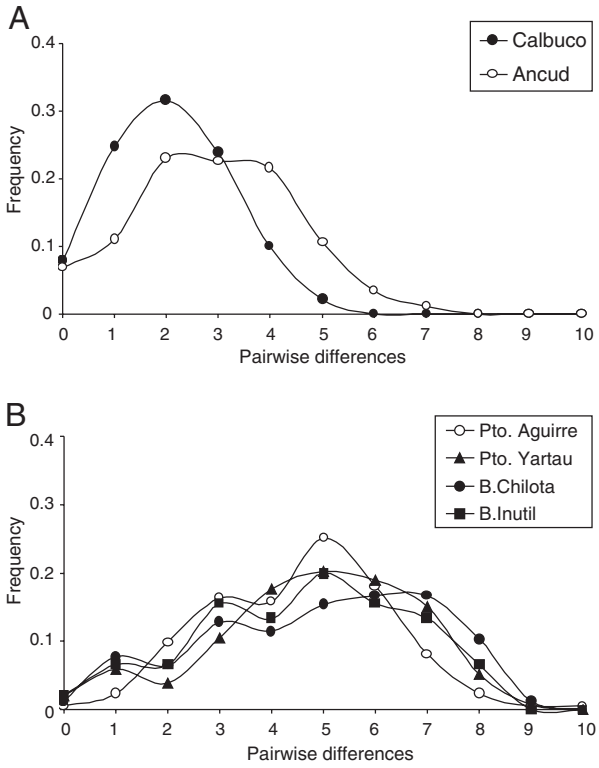

FIG. 2. Frequency distribution of pair-wise differences among haploid genotypes within (A) peripheral and (B) central populations. The lines connecting the data points are included only to highlight the distribution pattern of the differences for each locality, as only the integer values represent the true observed data.

TABLE 6. Pair-wise F_{ST} values calculated from allele frequency distribution.

	Calbuco	Ancud	Puerto Aguirre	Bahía Chilota	Bahía Inútil
Ancud	0.354 (55 km)				
Puerto Aguirre	0.489 (450 km)	0.478 (450 km)			
Bahía Chilota	0.490 (1000 km)	0.475 (1000 km)	0.453 (550 km)		
Bahía Inútil	0.540 (1000 km)	0.510 (1000 km)	0.414 (550 km)	0[a] (30 km)	
Puerto Yartau	0.570 (1000 km)	0.541 (1000 km)	0.413 (550 km)	0.190 (70 km)	0.247 (45 km)

Geographic distances between pairs of populations are indicated between brackets.
[a]Not significant.

few days (Santelices 1990). Reduced gene flow (i.e. low effective dispersal and recruitment among populations) has been confirmed by indirect estimates (based on molecular data) for several species of red algae (Engel et al. 1997, Wright et al. 2000, Faugeron et al. 2001, Zuccarello et al. 2001), where genetic differentiation has been detected at distances shorter than 1 km. This may explain the high F_{ST} values estimated in this study for most pair-wise comparisons considering that the distances among populations are important (30 km to more than 1000 km). The possibility of a reduced gene flow among northern peripheral populations of *G. skottsbergii* is further suggested by their low recruitment rates (Zamorano and Westermeier 1996). In *G. skottsbergii*, local genetic drift within marginal population is then exacerbated by its reduced dispersal abilities.

Our data strongly suggest the occurrence of a recent bottleneck in the two peripheral populations. The distributions of pair-wise differences among genotypes is skewed leftward (i.e. less pair-wise differences) for Calbuco and Ancud, with a higher proportion of shared genotypes than in each central population. Rogers and Harpending (1992) showed that such mismatch distribution indicates a demographic expansion, recently started from a small number of initial genotypes (i.e. after a bottleneck). Their model, however, was based on the distribution of nucleotide differences among DNA sequences resulting from mutation, whereas for our RAPD data, the assumption is that recombination is probably the main source of variation among haploid genotypes. In this context, the small number of pair-wise differences among genotypes in the two peripheral populations suggests that few recombination events occurred since the bottleneck. Nevertheless, additional evidence reinforces the hypothesis of a recent bottleneck in peripheral populations: a high proportion of shared genotypes and a reduced genetic diversity, particularly reduced allelic richness, which is more sensitive to bottlenecks. By contrast, in central populations we observed few shared genotypes, higher genetic diversity, and higher pair-wise differences among genotypes, all of which suggest that these populations are more demographically stable.

Demographic expansions, after a recent bottleneck, may be seen as part of a metapopulation process affecting marginal populations (Holt and Keitt 2000). However, Ray et al. (2003), by studying the intrademe genetic diversity in spatially expanding population at the border of a species range, showed that an excess of recent coalescent events is expected as compared with more central demes. Indeed, because of the genetic isolation of peripheral populations, genes would have more time to coalesce because they spend more time within the same deme (Ray et al. 2003). This suggests that inbreeding may be important and may explain why the populations of Calbuco and Ancud seem to have started a demographic expansion compared with central populations that appear to be near demographic equilibrium. On the other hand, we can also argue that because of genetic isolation (reduced gene flow), peripheral populations experienced the combined effects of frequent bottlenecks and recovery from local genotypes. Finally, whatever the explanation is, our data convincingly show that peripheral populations display a reduced genetic diversity and a recent demographic expansion.

The potential effects of overharvesting. Whether the differences between peripheral and central populations are due to the marginal conditions at the species' range boundary or to the population depletion because of overharvesting is difficult to determine. Overharvesting in this species has been assumed to occur on the basis of data indicating that harvested biomass included small plant sizes corresponding to infertile individuals (Avila et al. 1999, Westermeier et al. 1999). Marín et al. (2002) showed that this harvesting practice reduces the population biomass in a way that it does not allow the recovery to a condition similar to the preharvest level. This evidence may help to explain the population depletion observed in the northern populations (Avila et al. 2003), where landings almost completely ceased (SERNAPESCA 2001). In this context, the recent population expansion discussed earlier could be the consequence of a recent bottleneck due to overharvesting of the populations in Calbuco and Ancud. However, this hypothesis can hardly be tested. Indeed, because recombination is the main source of variation among RAPD genotypes, our data do not allow a precise timing of the origin of the demographic expansion. In addition, as all the marginal populations were already overharvested at the sampling date, it is difficult to discriminate between the two effects. In conclusion, the fact that Calbuco

and Ancud are marginal populations possibly made them more sensible to the strong harvesting pressure, just because their reproduction and recruitment are less efficient than in more central populations (Zamorano and Westermeier 1996). Then, overharvesting of these populations may have accentuated the deficit in genetic diversity, a characteristic of peripheral populations (as in Buchert et al. 1997).

Conservation perspectives. There is a vast literature on the ecological effects of harvesting on natural stocks (see Barilotti and Zertuche-González 1990, Schiel and Nelson 1990, Sharp and Pringle 1990, and Vásquez and Santelices 1990 for some reviews on changes in recruitment, survivorship and stability of harvested seaweed populations). Most of these studies led to management recommendations. Recently, Marín et al. (2002) examined the responses of marginal populations of *G. skottsbergii* in the Calbuco area to different management strategies using a simulation model. They recommended that commercial harvest should be based on selection for larger fronds (i.e. fertile fronds that could have already participated to the pool of new recruits) and restricted harvesting period. Their model simulates the population dynamics in terms of both number of fronds and biomass per size class, but their analysis was only performed in one peripheral site, with no comparison with central populations. To our knowledge, there is no example in the literature addressing population genetics aspects of depleted overharvested marine seaweeds. Only recently, the possible overexploitation of *Laminaria digitata* L. (Lamouroux) has been described as a serious risk for the gene flow among populations, the maintenance of the genetic diversity, and the sustainability of the resource (Billot et al. 2003). What follows is a brief discussion on the potential value of specifically conserving peripheral populations of *G. skottsbergii*.

When grown in relatively high temperature conditions, the better survival of adults and spores from the northern part of the geographic distribution compared with southern populations suggested the existence of ecotype differentiation (Bischoff-Bäsmann and Weincke 1996, Buschmann et al. 1999) between northern marginal and southern populations, separated by hundreds to thousands of kilometers. Surprisingly, the comparison of two populations located at the northern limit (Calbuco and Ancud), although only 54 km apart, also revealed differences in recruitment, survival, and frond growth rates (Westermeier et al. 1999). As pointed out by Lessica and Allendorf (1995), peripheral populations are under such contrasted ecological conditions that the selective pressures are highly heterogeneous and susceptible to drive adaptive differentiation, even among populations separated by short distances. If the genetic differentiation of the northern populations of *G. skottsbergii* is the result of local adaptation, as suggested by Bischoff-Bäsmann and Weincke (1996), then special care should be taken to characterize and preserve the genetic resource in this region. We would recommend, in this case, to implement harvesting strategies that at least allow the demographic recovery of the populations (Marín et al. 2002). Simultaneously, introductions of foreign genotypes (i.e. translocation of individuals to "reseed" overharvested populations), which would cancel the effects of local adaptation and destroy coadapted gene complexes (Jones et al. 2001), should be avoided.

Our results suggest that genetic drift may play an important role in the differentiation among peripheral populations and between peripheral and central populations. Under the isolation-drift scenario, inbreeding depression would be a major risk for the fitness of the genotypes of these populations. In this context, direct intervention would be required to increase local genetic diversity. It has been shown, however, that a reduced genetic diversity of small and peripheral populations is not necessarily correlated with a reduction of individuals' fitness and population viability (Lammi et al. 1999). The interest of these populations for conservation purposes would then rely mainly on their genetic uniqueness, and priority should be given to the preservation of these genetic pools.

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