

Population genetic structure and gene flow in the seagrass *Posidonia oceanica* assessed using microsatellite analysis

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ABSTRACT: Microsatellite markers were utilized in a study on population genetic diversity of the endemic Mediterranean seagrass *Posidonia oceanica* (L.) Delile. Five nuclear and one chloroplastic microsatellite markers detected low levels of polymorphism in 6 populations sampled along the coasts of Italy and Corsica (Western Mediterranean). The number of alleles per locus ranged from 1.5 to 2.0 and homozygosity was high within populations ($f = 0.314$). In the 120 individuals analyzed, only 32.5% were distinct genotypes. Although gene flow seems to exist between geographically disjunct meadows ($Nm = 1.55$), private alleles were found in some localities. In one population in particular (Lacco Ameno, Gulf of Naples), sampled at 2 different depths, a private allele was present only in the individuals of the shallow stand. Distance analysis identified genetic disjunction between the northern and the central-southern populations. This study indicates that (1) clonal growth is important in the maintenance of *P. oceanica* populations, (2) limited inbreeding occurs in *P. oceanica* populations, which can be composed of clonal patches of different size, (3) gene flow exists, but genetic disjunction between populations can be influenced by local forces, and (4) microsatellites are powerful markers in detecting genetic variability in clonally reproducing species.

KEY WORDS: Seagrass · Population genetics · Microsatellites · *Posidonia oceanica* · Clonal plants

INTRODUCTION

Seagrasses have colonized the marine environment apparently since the Cretaceous and have adapted to a completely submerged life cycle (Larkum & den Hartog 1989). They reproduce sexually or asexually, as occurs in terrestrial habitats, though they possess many structural and morphological modifications to accommodate for life in the sea (Philbrick & Les 1996). Among the seagrasses, different breeding systems have evolved, which do not reflect the level of intraspecific genetic diversity (Waycott & Les 1996). The genetic structure of seagrass meadows results from a combination of factors modulating seedling recruitment, extent of clonal propagation, and habitat physical features. Sexual reproduction allows for the introduction and inheritance of genetic variation, while asexual reproduction ensures clonal propagation

of successful genotypes, which are often adapted to stable environments (Les 1988). If dramatic environmental changes occur, clonal species can experience drastic reductions or extinction if they do not possess adequate genetic plasticity.

The seagrass *Posidonia oceanica* lives along the coasts of the Mediterranean Sea. Meadows extend from 3 to 40 m depth, according to light and nutrient availability (Duarte 1991). Sexual reproduction has been shown to be sporadic and seedling establishment is unsuccessful in the Western Mediterranean basin (Caye & Meinesz 1984, Thelin & Boudouresque 1985, Buia & Mazzella 1991, Meinesz et al. 1993). This is probably due to the stochastic nature of pollen and seed transport within and between populations, which is driven by both the current regime and local environmental forces (Caye & Meinesz 1984). Therefore, meadows should be composed of clonal patches, the expansion of which would depend upon the earlier and/or recent history of successful sexual reproductive

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events and seedling recruitment in a given locality. Moreover, apomixis and inbreeding by self-pollination within the same floral axis or between fragmented clonal patches can, together with clonal growth, contribute to the maintenance of low levels of genetic variability.

Previous studies performed using random DNA sequences and minisatellite regions showed an almost complete absence of genetic diversity within a *Posidonia oceanica* meadow and a very low genetic distance from other disjunct populations (Procaccini & Mazzella 1996, Procaccini et al. 1996). Low genetic variability in *P. oceanica* is also suggested by analysis of enzymatic polymorphisms (Capiomont et al. 1996). Due to the apparent high genetic uniformity of *P. oceanica* populations, the use of more polymorphic molecular markers is required to resolve the genetic diversity of this species. These markers must be capable of detecting polymorphism at the individual level and be scorable as individual neutral loci, with unambiguously identified codominant alleles. Microsatellite markers fit these requirements (Queller et al. 1993).

Simple sequence repeats (SSRs) or microsatellites (Weber & May 1989) have been shown to be highly variable genetic markers, present both in coding and non-coding regions of the genome (Charlesworth et al. 1994). The mutation rate of microsatellite loci is estimated to range between 10^{-2} and 5×10^{-6} (see Bruford & Wayne 1993 and Jarne & Ladoga 1996 for reviews). Despite the value of SSRs in population genetics (Bruford & Wayne 1993, Queller et al. 1993, Jarne & Ladoga 1996), few investigations to date have exploited this technology for population genetics of plants (see Powell et al. 1995, Byrne et al. 1996, Chase et al. 1996). Microsatellite analysis has never been applied to population genetics of aquatic plants.

In a previous analysis, microsatellite markers isolated from a *Posidonia oceanica* genomic DNA library were used to evaluate the existence of polymorphism for population studies (Procaccini & Waycott in press). Fourteen unambiguously scorable microsatellite regions were identified, 6 of which were polymorphic. The polymorphisms detected in *P. oceanica* populations (Procaccini & Waycott in press) were greater than those observed using minisatellite multilocus DNA fingerprints and RAPD markers (Procaccini & Mazzella 1996, Procaccini et al. 1996).

The aim of the work here was to explore the level of genetic diversity of *Posidonia oceanica* meadows in coastal waters of Italy and Corsica, with the goal of determining causes of the clonal structure of this species. This study is the first application of microsatellite markers to population genetics of marine vascular plants and opens the possibility of extending this approach to other seagrass species.

MATERIALS AND METHODS

Study sites and tissue collection. *Posidonia oceanica* (L.) Delile individual shoots were sampled in 6 Western Mediterranean populations along the coasts of Italy and Corsica (Fig. 1). Populations were located at Tonnara (Corsica, France), Vada (Tuscany coast, Italy), Ventotene Island (off the central Tyrrhenian coast, Italy), Lacco Ameno (island of Ischia, Gulf of Naples, Italy), Ieranto (Gulf of Naples, Italy), and Pantelleria Island (Sicily, Italy). All populations were distributed at 5 to 8 m depth, with the exception of Lacco Ameno (3 to 33 m depth), which was sampled at 2 different depths (5 and 22 m) and Ieranto (3 to 15 m depth). For each population, 20 shoots were collected at intervals of 7 m by SCUBA diving. Fresh tissue was transported to the laboratory, cleaned of epiphytes and stored frozen at -70°C for DNA extraction.

DNA isolation and microsatellite analysis. Genomic DNA was isolated from 1 g of frozen shoot tissue in CTAB buffer as in Procaccini et al. (1996). Six polymorphic loci [*Poc*-5: (TGG)₁₀; *Poc*-26: (GCGAGGA)₄; *Poc*-35: (TCC)₁₁; *Poc*-42: (TGC)₆; *Poc*-45: (TCC)₈(TTC)₄; *Poc*-trn: (TA)₅TTA(TA)₈TAAA(TA)₄] previously identified from a *Posidonia oceanica* genomic library (Procaccini & Waycott in press) were amplified in 120 individual shoots belonging to the 6 geographically disjunct populations. One of them (*Poc*-trn) was selected from an existing sequence of the *trnL* (UAA) chloroplast intron (Procaccini et al. in press). PCR amplification of microsatellite regions was performed in a 10 μl reaction volume with a ³²P labeled primer and alleles were separated on standard sequencing gels. The amplified SSR loci were run in a 48-well comb, which allowed processing of 2 populations at the same time, against a known nucleotide sequence used as a molecular-size marker.

Statistical analysis. The *Poc*-trn locus of the chloroplast DNA was not included in analysis of the heterozygosity and related statistics, as the chloroplast is maternally inherited in angiosperms (Hooper 1984).

Clonal diversity. Allelic bands resulting from the amplification of the 6 polymorphic loci were pooled to determine individual genotypic profiles. Genotypic diversity in *Posidonia oceanica* populations was calculated with the *G/N* ratio (Pleasants & Wendel 1989), where *G* is the number of distinct genotypes and *N* is the total number of samples.

Genetic variability and population structure: Statistical analysis was conducted taking into account that more than one ramet from the same population shared identical genotypes. Genetic parameters were hence calculated from analysis of only 1 ramet of each genotype per population (8, 8, 6, 12, 7 and 14 genotypes for Tonnara, Vada, Ventotene, Lacco Ameno, Ieranto and Pantelleria, respectively). Population genetic data

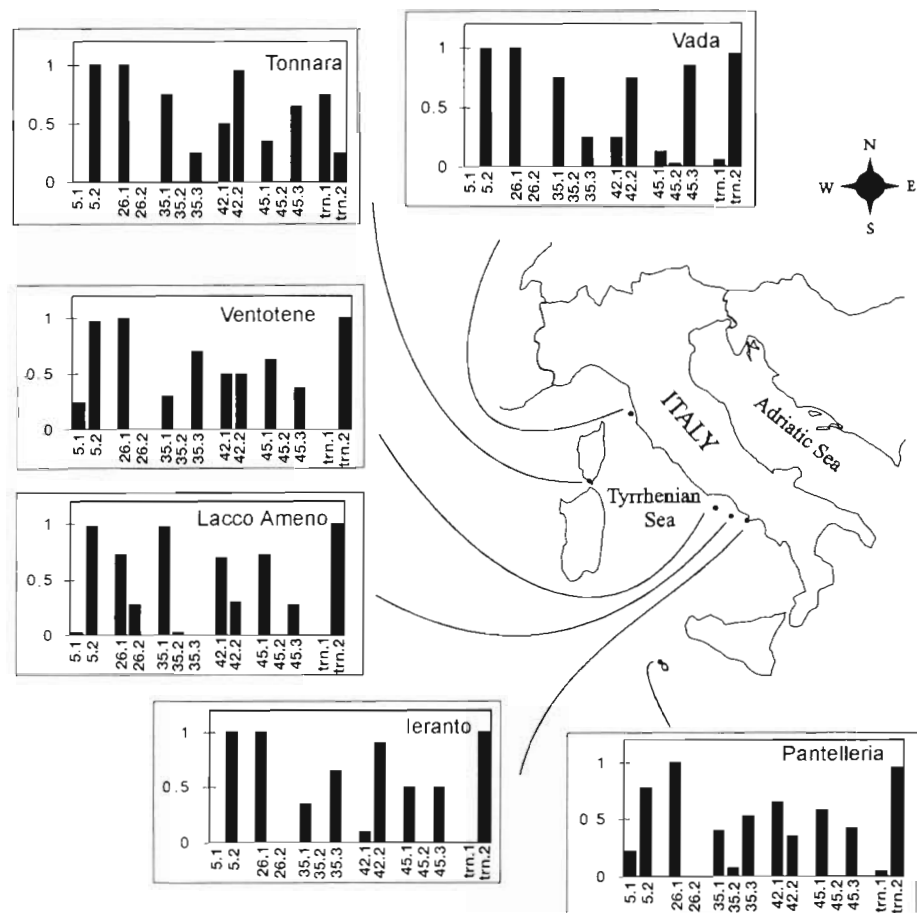


Fig. 1. Geographical position of the 6 *Posidonia oceanica* populations analyzed and allele frequency at each locality. Alleles at the same locus are numbered in sequential order from high to low molecular weight (see Table 1)

were calculated by using FSTAT (ver. 1.2, Goudet 1995). Weir & Cockerham's (1984) estimators of the level of inbreeding, within a population (f) and within the whole set of samples (F), were obtained. The 2 values are equivalent to Wright's (1951) F_{is} and F_{it} values, respectively, but should be unaffected by aspects of the sampling scheme (Weir & Cockerham 1984). The significance of f and F were assessed by a permutation test.

An Exact test of Hardy-Weinberg equilibrium was calculated for each population using the updated version of GENEPOP 1.2 (Raymond & Rousset 1995) computer package, through the complete enumeration method, as described by Louis & Dempster (1987).

Gene flow and genetic distance: Currently, there are different opinions concerning alternative methods for the assessment of genetic differentiation among populations with microsatellite markers. Valsecchi et al. (1997) found that different methods gave inconsistent results when applied to oceanic populations of humpback whales. In order to estimate genetic differentiation among populations, we calculated 2 statistical indices: θ (Weir & Cockerham 1984) and Rho (Goodman 1997), an unbiased estimator of Slatkin's R_{st} (Slatkin 1995).

The θ value is equivalent to Wright's F_{st} (1951) but, as for the above-mentioned f and F , should provide unbiased estimates of genetic disjunction among populations. Even more specific for microsatellite analysis is R_{st} . Nevertheless, R_{st} , while providing less biased estimates of population divergence assuming that microsatellites evolve by a step-wise mutation model (Slatkin 1995), still assumes populations of equal sample size and equivalent variances for all loci; the calculation of Rho deals with both sources of bias. The significance of θ and Rho was assessed by a permutation test. The number of migrants per generation was then calculated for θ (Nm) and for Rho (Nm^{Rho}) from the following relation: $F_{st}(\theta, Rho) = 1/(1 + 4Nm)$ (Wright 1943).

The allele-size variance method (D_1 in Goldstein et al. 1995) was then applied to calculate the genetic distances. For D_1 the distance values are a linear function of the separation time between 2 genotypes. D_1 distance values were used for a complete linkage clustering.

D_1 was calculated with the MICROSAT (Minch et al. 1995–1996) computer package. θ was calculated with FSTAT (Goudet 1995). Rho was calculated with an RST Calc package (Goodman 1997).

RESULTS

Fourteen alleles were present in the 6 *Posidonia oceanica* populations analyzed (Table 1). Allele frequency distribution across the 6 polymorphic loci did not show the same pattern in the different populations (Fig. 1, Table 1). Common alleles were present with

high frequencies in all populations (e.g. alleles 5.2 and 26.1 in Fig. 1, Table 1), or with different frequencies not consistent with the geographical distribution (e.g. alleles 35.1, 42.2 and *trn.2*; Fig. 1, Table 1). Rare or private alleles were present in some populations. In the Lacco Ameno population, in particular, *Poc*-26.275 was common and unique to all the individuals collected at -5 m depth (10 individuals), while it was not present in the 10 individuals collected at -22 m depth (Figs. 1 & 2). The other unique allele (*Poc*-45.165) was present only in 1 individual in the Vada population (Fig. 1, Table 1).

Table 1. *Posidonia oceanica*. Allele frequencies for the 6 polymorphic microsatellite loci in 6 populations. For sequence and size of microsatellite regions see Procaccini & Waycott (in press)

Locus and allele	Tonnara	Vada	Ventotene	Lacco Ameno	Ieranto	Pantelleria
<i>Poc</i> -5						
.176	0.000	0.000	0.025	0.025	0.000	0.225
.173	1.000	1.000	0.975	0.975	1.000	0.775
<i>Poc</i> -26						
.282	1.000	1.000	1.000	0.725	1.000	1.000
.275	0.000	0.000	0.000	0.275*	0.000	0.000
<i>Poc</i> -35						
.200	0.750	0.750	0.300	0.975	0.350	0.400
.197	0.000	0.000	0.000	0.025	0.000	0.075
.194	0.250	0.250	0.700	0.000	0.650	0.525
<i>Poc</i> -42						
.176	0.050	0.250	0.500	0.700	0.100	0.650
.170	0.950	0.750	0.500	0.300	0.900	0.350
<i>Poc</i> -45						
.168	0.350	0.125	0.625	0.725	0.500	0.575
.165	0.000	0.025*	0.000	0.000	0.000	0.000
.144	0.650	0.850	0.375	0.275	0.500	0.425
<i>Poc-trn</i>						
.409	0.750	0.050	0.000	0.000	0.000	0.050
.395	0.250	0.950	1.000	1.000	1.000	0.950

*Private alleles

The percent of polymorphic loci in each population ranged from 50% at Ieranto to 83.3% in the Lacco Ameno and Pantelleria Island populations (Table 2). The number of alleles per locus was low, with only 2 loci possessing more than 2 alleles. The mean values in different populations ranged from 1.5 (Ieranto) to 2.0 (Pantelleria Island) (Table 2). All populations were represented by more than 1 clone. In the total 120 ramets, 39 genets were present, some of which were shared by more than 1 population. The overall *G/N* value was 0.325. The *G/N* values for individual populations (expression of the likelihood of new clones within a population) ranged from 0.30 at Ventotene (6 genotypes) to 0.70 at Pantelleria (14 genotypes; Fig. 3). The number of unique genotypes varied in the 6 populations (Fig. 3), and only 1 genotype, characterized by the presence

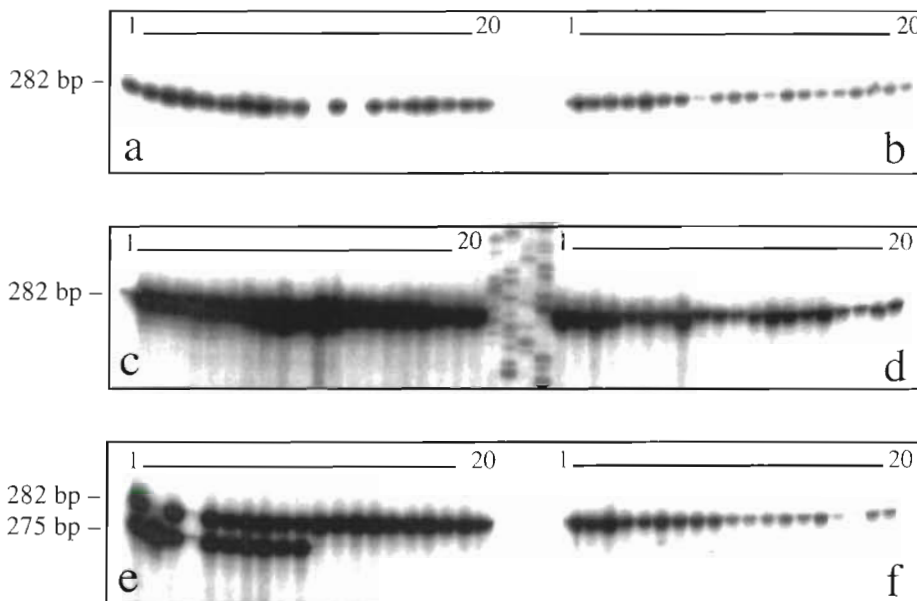


Fig. 2. *Posidonia oceanica*. Amplification of the microsatellite region *Poc*-26 from the 120 individuals belonging to the 6 populations analyzed. (a) Tonnara. (b) Vada. (c) Ventotene. (d) Ieranto. (e) Lacco Ameno. (f) Pantelleria. The low molecular weight *Poc*-26.275 allele is present only in the first 10 individuals (collected at 5 m depth) of Lacco Ameno. Nucleotide sequence, used as molecular weight marker, is shown only in the middle panel

Table 2. *Posidonia oceanica*. Genetic variation averaged over 6 polymorphic microsatellite loci in 6 populations. Pantelleria is the only population not in Hardy-Weinberg equilibrium

Population	Sample size	No. of distinct genotypes ^a	Mean no. of alleles per locus	Percentage of polymorphic loci	Expected heterozygosity ^b	H-W equilibrium ^b (p-value)
Tonnara	20	8	1.7 (0.2)	66.7	0.190 ± 0.147	0.14
Vada	20	8	1.8 (0.3)	66.7	0.207 ± 0.157	0.80
Ventotene	20	6	1.7 (0.2)	66.7	0.295 ± 0.202	0.32
Lacco Ameno	20	12	1.8 (0.2)	83.3	0.270 ± 0.190	0.68
Ieranto	20	7	1.5 (0.2)	50.0	0.233 ± 0.170	0.18
Pantelleria	20	14	2.0 (0.3)	83.3	0.380 ± 0.246	<0.001

^aSome genotypes can be present in more than one population (see Fig. 3)
^bOnly for the 5 nuclear loci. Values were calculated for distinct genotypes

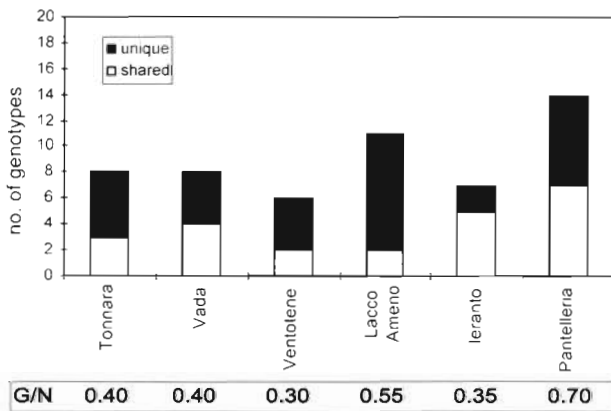


Fig. 3. *Posidonia oceanica*. Total number of genotypes in the 6 populations. The number of unique genotypes and the number of genotypes present also in other localities are indicated in black and in white, respectively. The G/N values (percent of genotypes in total number of samples) are also given

of 8 alleles, was present in all populations. Four populations (Tonnara, Vada, Ventotene and Ieranto) were more homogeneous, with only 22 genotypes represented ($G/N = 0.27$). Sampling in this group of 4 populations resulted in only a 27% possibility of finding new genotypes. These populations were characterized, in some cases, by groups of 3 or 4 spatially close individuals belonging to the same genotype classes.

Expected heterozygosity values were low, varying within a narrow range, and differences were not significant (Table 2). The individuals of the Tonnara population showed the lowest levels of heterozygosity (0.190), while the southernmost population of Pantelleria showed the highest values (0.380). Pantelleria was the only population not in Hardy-Weinberg equilibrium, calculated from the average of all loci.

The mean value of f was positive (0.314; Table 3), confirming homozygosity excess within populations. The F value was even greater (0.411; Table 3), demon-

strating that homozygote excess is more evident in the compound population than in the single population units.

The mean value of θ calculated only for nuclear loci was 0.139 (Table 3), showing the existence of genetic differentiation among the 6 populations. The Rho value (0.213; Table 3), even greater than θ , confirmed the data.

The 2 estimates of the mean number of migrants per generation (Nm and Nm^{Rho}) among the sampled *Posidonia oceanica* populations were 1.55 and 0.925 respectively, showing the existence of moderate gene flow.

The values obtained for genetic distance using the allele-size variance method (D_1 in Goldstein et al. 1995; Table 4), where the distance values are directly related to the separation time between 2 genotypes, were con-

Table 3. *Posidonia oceanica*. Estimates of f , F and θ (Wright's F_{is} , F_{it} and F_{st} , respectively) Rho , Nm and Nm^{Rho} for the polymorphic nuclear loci in the 6 populations

f	F	θ	Rho	Nm	Nm^{Rho}
0.314*	0.411*	0.139*	0.213*	1.55	0.925

*Significant values ($p \leq 0.001$)

Table 4. *Posidonia oceanica*. D_1 (Goldstein et al. 1995) distance matrix for 6 populations

	Tonnara	Vada	Ventotene	Lacco Ameno	Ieranto
Vada	3.228				
Ventotene	4.856	3.392			
Lacco Ameno	4.591	2.793	0.367		
Ieranto	3.612	0.740	1.258	1.058	
Pantelleria	3.377	2.177	0.213	0.381	0.583

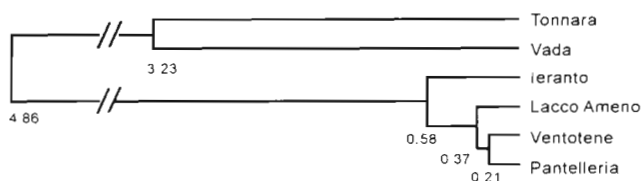


Fig. 4. *Posidonia oceanica*. Maximum linkage dendrogram of the 6 populations based on the D_1 distance values (Goldstein et al. 1995) of Table 4. Distance values are indicated for each branch

sistent with the geographical pattern. Values among the 4 central-southern populations ranged from 0.213 to 1.258, while the distances between these and the 2 northern populations ranged from 0.740 to 4.856. Tonnara appears to be the most isolated population among those examined. The complete linkage dendrogram of the Goldstein's distance shows populations grouped in 2 main clusters following a latitudinal gradient. Tonnara and Vada are clearly distinct, followed by the other 4 populations (Fig. 4). The *Poc-trn* locus generated the highest distance values, isolating the population of Tonnara, where the allele *Poc-trn*.409 was present with a frequency of 0.75. The other populations showed a maximum frequency of only 0.050 for this same allele (Figs. 1 & 5, Table 1).

DISCUSSION

Our analysis detected only low levels of polymorphism in 6 populations of the seagrass *Posidonia oceanica* from the Western Mediterranean Sea. The number of alleles per locus in the 6 polymorphic loci used in the analysis ranged from 2 to 3. These values are much lower than those reported in a broad range of terrestrial plants where the number of alleles per locus is as high as 26 (e.g. soybean; Rongwen et al. 1995). The finding here of only 14 alleles in total for 6 loci indicates that microsatellite polymorphism among the 120 *Posidonia oceanica* individuals analyzed in this study is very low for vascular plants.

Although not always statistically significant, the differences in genetic diversity parameters among populations, shown in Table 2, allow some discussion.

The number of genets varied among the different populations, indicating that local forces affect population genetic variability. The Pantelleria population showed the highest genetic diversity, with a large number of alleles per locus and a high percent of polymorphic loci and genotypes. These results suggest that sexual reproduction occurs regularly in this population, which is consistent with the observations of regular flowering and fruiting in populations growing along the coasts of Sicily and Pantelleria (pers. obs.).

In the Lacco Ameno population the large number of genotypes found, 80% of which were exclusive to this population, is attributed to the presence of the private allele *Poc*-26.275 in the 10 individuals sampled at the 5 m depth. The presence of an exclusive allele suggests genetic subdivision of this population. The habitat for this population is characterized by a steep depth gradient with different depth-dependent physical (thermocline at 15 m depth) and biological features (e.g. temporal shifts in flower and fruit development) (Buia & Mazzella 1991). We hypothesize that different timing in the maturation of gametes between the shallow and deep portions of the populations acts as a physiological barrier leading to genetic isolation, and thus the creation of 2 sub-populations. Male gametes produced in the shallow region are probably flushed away from the bed before the female flowers of the plants in the deep portion are mature. The stochastic nature of seedling establishment in this population serves to increase the genetic disjunction between the 2 subpopulations. It is interesting to note that the *Poc*-26.275 allele was also detected previously (Procaccini & Waycott in press) in individuals collected at the same depth in San Pietro, a location only 5 km from Lacco Ameno on the coast of Ischia. The only other population sampled at 2 different depths (Ieranto) did not show a similar depth-dependent genetic pattern; however, it is unknown whether significant gradients in physical features exist at this location.

The 4 *Posidonia oceanica* populations represented by lower numbers of genets [Ventotene (30%), Ieranto (35%), Tonnara (40%) and Vada (40%)] were characterized by a patchy distribution, with patches separated by large inter-'matte' channels. Whether the microstructure of these meadows is related to the genetic structure of the populations is unknown and

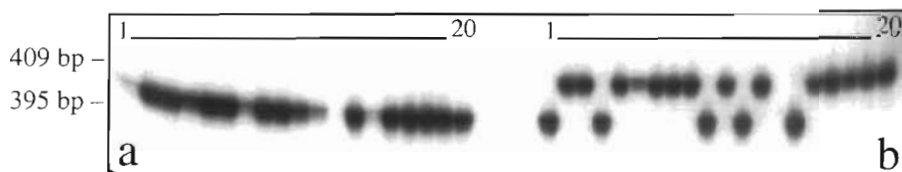


Fig. 5. *Posidonia oceanica*. Amplification of the microsatellite region *Poc-trn* from a panel of individuals belonging to (a) Lacco Ameno and (b) Tonnara populations. Lacco Ameno individuals only possess the *Poc-trn*.395 allele, while *Poc-trn*.409 is present in many individuals in Tonnara

requires further investigation. Our analysis showed that spatially close individuals can be genetically identical, indicating that each physical patch probably constitutes a single genet. Considering that individual shoots were sampled at distances of 5 to 7 m, clonal patches of at least 20 m in diameter are likely. If this is the case, the populations appear to be composed of a number of old genets, growing clonally, with little or no exchange of sexual products.

The mean value of f obtained in the present study indicates a 31.4% excess of homozygosity. The high level of homozygosity can either result from pooling together genetically distinct groups (Wahlund Effect) or may reflect limited inbreeding within populations, and may be related to the fact that *Posidonia oceanica* is monoecious with bisexual inflorescences. To date, however, it is not clear whether self-compatibility exists in this species. Nevertheless, in *P. oceanica* clonal reproduction appears to be dominant (Caye & Meinesz 1992, Procaccini et al. 1996) and meadows appear to be structured by clones of different size (Caye & Meinesz 1992; see below) which can also be fragmented by overgrowth of different genotypes. In this scenario, it is possible that *P. oceanica* is self-compatible in respect to a separated shoot belonging to the same clone, and that a single genotype can spread within a meadow by sexual reproduction, as suggested by other investigators (Caye & Meinesz 1992). In the congeneric species *P. australis*, self-compatibility is suggested based upon observed levels of inbreeding (Waycott & Sampson 1997).

The differences among populations in heterozygosity levels and in the expectation of Hardy-Weinberg equilibrium could be the result of different levels of inbreeding due to the local water flow regimes. Pollen transport within and external to a meadow is directly related to the amount of water flow and to the direction and intensity of the dominant currents in specific areas (Ackerman 1986, Ruckelshaus 1996). Considering that seedlings are rarely observed in some populations and that at Lacco Ameno, in particular, seedlings have not been reported in the last 15 yr (Buia & Mazzella 1991, pers. obs.), our data on the genetic structure of the meadows most probably reflect events that occurred in the past and the current level of homozygosity is a result of historical pollen transport phenomena.

Gene flow is moderate among the *Posidonia oceanica* populations examined here. The 2 estimates of the mean number of migrants per generation ($Nm = 1.55$, $Nm^{Rho} = 0.925$) are both close to the threshold of 1.0, a value that represents the minimum for avoiding significant differentiation among populations (Slatkin 1985). The comparison with the values suggested by Slatkin, however, could be affected by the presence of shared genotypes between populations; nevertheless, both θ

and Rho estimates of genetic differentiation show that populations are genetically distinct. Thus, our results suggest that the gene flow among genetically distinct populations is probably determined by sporadic and haphazard transport of sexual products or vegetative propagules. The existence of identical genotypes in different localities can also be attributed to population fragmentation due to a loss of original habitat, caused by natural events or by human impacts. Since to date no data exist on the dispersal distance of *P. oceanica* sexual products, both hypotheses should be considered.

The average square distance method used here showed that the 2 northernmost populations are genetically disjunct. Considering that this distance is directly related to the separation time (e.g. number of generations) between 2 genotypes (Goldstein et al. 1995), the genetic isolation of the northern genotypes from the other populations appears to be ancient.

The finding that the chloroplastic locus (*Poc-trn*) provides the main contribution to the genetic disjunction of Tonnara from the other populations is in accord with the theory predicting that uniparentally inherited haploid loci should exhibit the highest variation among populations (Birky et al. 1983).

Physical barriers to gene flow can account for genetic disjunction, and the impact of physical features such as currents and local hydrodynamic regimes can influence seed and pollen dispersal. Eckman (1987) showed that mean flow within *Zostera marina* beds was significantly slower than that outside of the meadow, while Ackerman (1986) showed that pollen dispersal was significantly impacted by canopy structure (shoot density and height) and flow regime within the *Z. marina* beds. The apparent lack of short distance (<10 km) water transport of *Z. marina* seeds between populations within the same Bay (Fain et al. 1992, Alberte et al. 1994) and the possible role of water flow in long distance transport have been identified (Alberte et al. 1994) as factors contributing to genetic isolation in seagrass populations in the United States.

This is the first report of microsatellite genetic analysis applied to clonally reproducing plants. We show that populations of *Posidonia oceanica* in the Western Mediterranean basin possess nuclear and chloroplastic microsatellite regions that are useful molecular markers for detecting polymorphism in this species, where little to no genetic diversity has previously been reported (Procaccini et al. 1996). Nevertheless, the variability detected from SSR loci is still low, suggesting that a larger number of samples should be used in future studies. In fact, *P. oceanica* meadows appear to be composed of clonal patches of different size and, when sexual reproduction occurs, limited inbreeding can result. Populations of *P. oceanica* are genetically

disjunct, with private alleles characterizing some populations. The level of gene flow between populations seems to be related to geographical distance and the existence of physical barriers between meadows. The low genetic variability of *P. oceanica* revealed here is not a common feature of the *Posidonia* genus, as high levels of genetic diversity have been observed in *P. australis* populations along the Western coast of Australia (Waycott 1995, 1996).

That vegetative propagation is the dominant reproductive mode of *Posidonia oceanica* has implications for the conservation and management of *P. oceanica*-based ecosystems in the Mediterranean Sea, where strong anthropogenic pressure serves to enhance the negative impact of local environmental and global climatic changes (Boudouresque et al. 1989). Future studies will be required to assess the short- and long-term impacts of environmental change on the stability and genetic structure of seagrass populations worldwide as these systems serve as buffers of land-based human activities.

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