Spatial patterns of genetic diversity in *Posidonia oceanica*, an endemic Mediterranean seagrass

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Abstract

Posidonia oceanica is an endemic seagrass species in the Mediterranean Sea. In order to assess levels of genetic structure in this species, the microsatellite polymorphism was analysed from meadows collected in several localities, along the coasts of the Tyrrhenian Sea (Mediterranean Sea). The existence of single population units and the recruitment of seedlings collected in some localities were investigated. Moreover, genetic structure at different spatial scales and biogeographic relationships among populations were also assessed. Our analysis showed the existence of clear patterns of genetic structure in *P. oceanica* in the area considered in the analysis. *P. oceanica*, in fact, is present in separate meadows that represent discrete populations, characterized by low genetic diversity. Comparable levels of genetic variability between mature meadows and seedlings were found. Patterns of genetic relatedness among populations seem to be in accord with direction of dominant current flux in the whole area, separating South Tyrrhenian from North Tyrrhenian populations. Moderate levels of gene flow between populations and genetic substructure within populations, together with the finding of the limited role of sexual reproduction in increasing genetic variability, should be a cause for concern for the persistence of this essential resource in the Mediterranean basin.

Keywords: genetic diversity, genetic structure, microsatellites, MRPP, population, *Posidonia*

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Introduction

Most of the spatial genetic diversity of species in natural landscapes has a hierarchical structure (Avice 2000). Overall genetic diversity is structured into within-population and among-populations components, and is essentially related to the amount of gene flow (Slatkin 1987). Extensive gene flow can prevent genetic isolation and fixation of genetic differences among populations, according to the dispersal capability of a given species and to different characteristics of the landscape (Amos & Harwood 1998; Dieckmann *et al.* 1999; Sork *et al.* 1999). Existence of natural barriers and human driven habitat fragmentation can enhance the isolation of natural populations, with the consequence of reducing gene flow among localities. In order to understand within species patterns of evolution and species persistence and expansion in natural environments, the

study of genetic variability and population differentiation is of fundamental importance. As such, this becomes an important factor to consider in the management of a natural resource and for the conservation of the evolutionary potential of a given species (Meffe & Carroll 1997).

Seagrass meadows represent important coastal ecosystems worldwide, and have been considered as high-value natural ecosystems, even compared with terrestrial habitats (Costanza *et al.* 1997). Population genetic studies in seagrasses have often revealed unpredictable patterns of genetic diversity, not always related to reproductive system and dispersal potential (Waycott & Les 1996; Waycott *et al.* 1997; Procaccini & Mazzella 1998; Waycott 1998; Procaccini *et al.* 1999; Reusch *et al.* 2000). Local environmental conditions and geological history seem to be the main factors in determining population genetic structure in this group of marine organisms. Different populations of the same species, in fact, can go from being uniclinal to being extremely genetically diverse, in relation to history of patch colonization and dynamics in different geographical areas (Reusch *et al.*

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1998; Waycott 1998). Meadow structure is the result of a range of different factors modulating seedling recruitment, extent of clonal propagation and habitat physical features. As such, the genetic make up of populations derives from the balance between sexual reproduction and vegetative propagation, whose reciprocal importance varies among species and localities. Meadows or patches of different size can be represented by a variable number of genotypes and the assessment of population boundaries is often difficult.

Posidonia oceanica (Posidoniaceae), an endemic seagrass species in the Mediterranean sea, forms extensive monospecific meadows along the coasts of the whole basin. This species is extremely important to a wide range of human activities in the Mediterranean Sea, such as coastal fisheries and tourism and is extremely sensitive to moderate-to-high disturbance often associated with highly human-impacted coasts (Marbà *et al.* 1996; Short & Wyllie-Echeverria 1996).

Posidonia oceanica is characterized by long persistence, slow vegetative growth, sporadic sexual reproduction and low genetic variability (Buia & Mazzella 1991; Buia *et al.* 1992; Mateo *et al.* 1997; Procaccini & Mazzella 1998). Our previous studies of *P. oceanica* population genetics were conducted on meadows geographically isolated along the Western Italian coasts (Procaccini & Mazzella 1998) with the goals of inferring overall genetic variability and gene flow among localities; precise assessment of population distinction was not carried out at finer geographical scales. Recent results obtained for other seagrass species indicate that genetic diversity can be structured at different spatial scales (Procaccini *et al.* 1999; Reusch *et al.* 2000) but a precise definition of genetic structure at different hierarchical levels in most cases is still lacking. The definition of single population units and the identification of genetic substructuring at different scales, from landscapes to populations are essential components in the identification of strategies for protection and management of this important marine system and for natural resources in general (Loeschcke *et al.* 1994; Meffe & Carroll 1997).

In order to define levels of genetic structure in *Posidonia oceanica*, we analysed microsatellite polymorphism of meadows collected in several localities, from the Ligurian coasts (North Tyrrhenian Sea) to Pantelleria (Sicily channel). We investigated the existence of single population units and the recruitment of seedlings collected in some localities. Moreover, we also assessed genetic structure at different spatial scales and biogeographic relationships among populations.

Materials and methods

Sampling sites

Almost 700 *Posidonia oceanica* shoots were collected in 17 localities following a latitudinal gradient along the Western coasts of Italy (Fig. 1). In three localities (Meloria, Follonica

Fig. 1 Geographic location of sampling sites. Localities where both seedlings and mature meadows have been collected are indicated with an asterisk. CA = Camogli; GO = Gorgona; VA = Vada; EL = Elba; ME = Meloria; FO = Follonica; CI = Civitavecchia; TO = Tonnara; TA = Tavolara; VE = Ventotene; IS-3 = Ischia-LA (Lacco Ameno), Ischia-CDI (Cava dell'Isola), Ischia-PC (Punta Caruso); IE = Ieranto; PI = Pioppi; MI = Milazzo; PA = Pantelleria.

and Vada) patches of seedlings established *in situ* near mature meadows were also sampled. One isolated patch of seedlings found *in situ* in 1999 was also collected at one locality (Ischia-PC). In seven meadows samples were collected both below and above 15 m depth, to verify the existence of the depth-related genetic structure found in a previous analysis (Procaccini & Mazzella 1998). In four of the six localities where 20 shoots had already been sampled and analysed (Vada, Ventotene, Ischia-LA, Pantelleria; Procaccini & Mazzella 1998) the number of samples was increased to confirm the indication of low genetic polymorphism. Almost 40 individual shoots were collected in total for each meadow, at the reciprocal distance of 7–10 m, trying to minimize the risk of sampling within the same clonal patch.

Microsatellite analysis

DNA extraction was performed in hexadecyltrimethyl ammonium bromide (CTAB) buffer and microsatellite regions were amplified and visualized as in Procaccini & Mazzella (1998). Six polymorphic microsatellite regions, five nuclear and one chloroplast (Procaccini & Mazzella 1998; Procaccini & Waycott 1998), were utilized in the analysis.

Multilocus genotypes were assessed for each individual ramet and overall genetic diversity was calculated as the per cent of different genotypes over the total number of sampled ramets (G/N , Pleasant & Wendel 1989). To investigate the possibility that two identical genotypes are not truly clone-mates we calculated both the theoretical number of different genotypes within the entire data set according to Reusch *et al.* (1998), i.e.

$$N_g = \prod_{i=1}^L [a_i(a_i+1)] / 2$$

and the P_{gen} value for single genotypes in each sampling location (Parks & Werth 1993; Reusch *et al.* 1998), i.e.

$$P_{\text{gen}} = \prod_{h=1}^k (p_i) \cdot 2^h$$

For genotypes with $P_{\text{gen}} > 0.05$ we cannot exclude the possibility that identity results from random recombination of alleles.

Definition of population units and population subdivision

The existence of discrete population units was tested utilizing two different approaches based on resampling statistics. In the first case a multi-response permutation procedure (MRPP; Zimmerman *et al.* 1985) was utilized. This is a nonparametric permutation procedure for testing the hypothesis of no difference between two or more a priori defined groups. It compares the observed mean within-population Euclidean distance with the expected value given the null hypothesis that all the data came from a single population. Significance was assessed by means of Student's *t*-test (the Exact test is not feasible). In the second case, the observed mean (between-population) ($\delta\mu$)² distance (Goldstein *et al.* 1995) was compared to the empirical distribution of the same distance that was obtained after 10 000 permutations of the data matrix, i.e. after randomly distributing 10 000 times the observations among samples (given their original size) and computing the resulting ($\delta\mu$)² distance. Different analyses were independently performed: (i) to test the hypothesis of identity between seedlings and mature meadows sampled in the same site; (ii) to test the existence of discrete population units, considering only mature meadows; and (iii) to test the existence of depth-related subgroups within populations.

Within-population genetic diversity

P. oceanica meadows are composed of clonal patches that differ in size. Although sampling was designed to minimize the possibility of collecting within the same clone, identical genotypes were found within the same population

and some of them were shared among different meadows. In order to assess the amount of genetic polymorphism within separated meadows we calculated the G/N (Pleasant & Wendel 1989) and D_G (Ellstrand & Roose 1987) values for each population. The following population genetic parameters were calculated on the data obtained from the five nuclear loci (Procaccini & Mazzella 1998) utilizing only one ramet of each genotype per population: Weir & Cockerham's (1984) estimator of the level of inbreeding — f — (using FSTAT ver. 2.9.1, Goudet 2000), the significance of which was assessed by a permutation test; exact test of Hardy–Weinberg equilibrium, through the probability test (using GENEPOP 3.1d, Raymond & Rousset 1995). In order to verify the independence of all loci we tested for linkage disequilibrium between all pairs of loci using GENEPOP 3.1d (Raymond & Rousset 1995).

Biogeographic trend and gene flow

Gene flow was assessed calculating ρ , θ and the related Nm values [from the following relation: $F_{ST}(\theta, \rho) = 1/(1 + 4Nm)$; Wright (1943)] among all sampled meadows. To assess phylogeographic relationships among populations we calculated ($\delta\mu$)² distance value and a distance matrix was utilized for a nonmetric multidimensional scaling (MDS). MDS was used because it can show complex patterns of genetic relatedness not detected by the traditional cluster analysis (Lessa 1990). Values of ρ and ($\delta\mu$)² were calculated using RST CALC (Goodman 1997); θ was calculated with FSTAT ver. 2.9.1 (Goudet 2000); MDS was performed using the STATISTICA (version 5.0; StatSoft) computer package.

Results

In general, the amount of genetic diversity of *Posidonia oceanica* was low in the 17 localities where 20 meadows have been sampled. Only 19 alleles were found in total, with a maximum of six alleles in the locus *Poc-35* (Table 1). No evidence of linkage disequilibrium among loci was

Table 1 Total number of alleles for the six microsatellite loci and estimates of Hardy–Weinberg equilibrium (HWE) and homozygosity (f) for the five nuclear loci utilized over the total number of samples

Loci	No. of alleles	HWE	f
<i>Poc-5</i>	2	NS	0.198**
<i>Poc-26</i>	3	NS	0.187**
<i>Poc-35</i>	6	**	0.651**
<i>Poc-42</i>	2	NS	0.161**
<i>Poc-45</i>	3	NS	0.213**
<i>Poc-trn</i>	3	—	—

NS = not significant; ** $P < 0.01$.

Table 2 MRPP test for identity of a priori defined groups. Both the observed and the expected mean within-group Euclidean distances, as well as the *P* level, are shown

	Observed	Expected	<i>P</i>
Mature meadows vs. seedlings			
Meloria	24.34	24.43	NS
Vada	17.18	17.18	NS
Follonica	24.52	25.02	*
Comparison among mature meadows only			
	19.20	23.95	**
Shallow vs. deep stands			
Meloria	21.59	23.06	**
Vada	15.10	15.22	NS
Follonica	23.59	25.58	*
Elba	16.46	19.89	**
Tavolara	15.61	20.02	**
Ischia-LA	16.85	19.03	*
Pantelleria	15.88	18.67	**

NS = not significant; **P* < 0.05; ***P* < 0.01.

found, according to the Fisher's global test, across all populations (*P* not significant for all pairings of loci). Only one of the nuclear loci (*Poc-35*) was not in Hardy-Weinberg equilibrium (Table 1). Eighty-one multilocus genotypes were found over the 699 ramets analysed, giving a total sample *G/N* value of 0.116. The number of possible different genotypes within the entire data set (*N_g*) was 40824, thus indicating that only the 0.19% of the possible genotypes was detected.

Definition of population units

Results of the two statistical approaches utilized to test the genetic identity of a priori defined groups are shown in Tables 2 and 3 and in Fig. 2. In the light of the MRPP test the seedling patches were not different from the mature meadows sampled in the same locality in two out of three

Table 3 Observed (Observ.) mean ($\delta\mu$)² distances, lower (Min.) and upper (Max.) limits of their empirical distribution after 10 000 permutations of the data matrix and *P* level estimates based on the rank of the observed distance within the empirical distribution

	Observ.	Min.	Max.	<i>P</i>
Mature meadows vs. seedlings				
Meloria	0.692	0.002	3.821	NS
Vada	0.576	0.002	2.844	NS
Follonica	1.60	0.004	6.834	NS
Comparison among mature meadows only				
	5.891	0.315	1.718	**
Shallow vs. deep stands				
Meloria	6.154	0.018	9.092	**
Vada	0.371	0.010	2.787	NS
Follonica	8.458	0.017	12.348	*
Elba	7.447	0.071	7.602	**
Tavolara	2.095	0.042	6.360	NS
Ischia-LA	4.254	0.002	7.707	*
Pantelleria	5.437	0.049	6.412	**

NS = not significant; **P* < 0.05; ***P* < 0.01.

cases if *P* = 0.05, whereas the null hypothesis (identical genotypes) was never rejected if *P* = 0.01 (Table 2). This result was confirmed by the second statistical approach, where the observed ($\delta\mu$)² value fell in the central part of the empirical distribution of ($\delta\mu$)² values obtained after 10 000 permutations of the data matrix (*P* not significant; Table 3). According to such a result, seedling patches and mature meadows cannot be considered as independent populations. In order to test the independence of the meadows sampled in different localities, data from seedling patches were not considered. For each mature meadow, the MRPP test compared the observed mean values of within-groups Euclidean distances to the mean expected values. Only in two cases (Gorgona and Follonica) single population values were higher than expected (Fig. 2), while average observed values were significantly lower than expected (*P* < 0.01, Table 2). The independence of single population units was

Fig. 2 MRPP test among mature meadows. The expected mean within-meadow Euclidean distance is shown in the box. Only in the Gorgona and Follonica meadows the observed values were higher than expected (i.e. the genetic diversity was higher than expected).

confirmed by the between-population permutation test based on $(\delta\mu)^2$ distance. The observed value, in fact, was considerably higher than the maximum value in the empirical distribution of the resampled mean $(\delta\mu)^2$ distances ($P < 0.01$; Table 3).

Within-population genetic diversity and substructuring

P_{gen} values were higher than 0.05 in 30 of the 81 genotypes found. These were distributed over 11 populations, varying from one to five per population (data not shown). The proportion of polymorphic loci ranged from 0.33 in Ischia-CDI to 0.83 in Pantelleria (Table 4). The number of alleles per locus was low, with an average of 1.76 (ranging from 1.3 to 2.2; Table 4). Different populations were represented by different number of clones, with values of G/N ranging from 0.16 in Ischia-CDI (six genotypes) to 0.54 in Pantelleria (21 genotypes) and values of D_G ranging from 0.52 in Ischia-CDI to 0.96 in Vada S (Table 4). The number of exclusive genotypes also varied, with the highest values in Ischia-LA and Pantelleria, where seven different genotypes were present only in each of the two localities.

Observed heterozygosity values were low, with an average of 0.178 and ranging from 0.057 in Gorgona to 0.340 in Ischia-LA. Only in two cases (Ischia-LA and Milazzo) observed heterozygosity values were higher than expected. Homozygosity values (f) calculated for single meadows had a wide range, from -0.091 in Ischia-CDI to 0.802 in Gorgona. Overall, 40% of meadows (Camogli, Meloria S, Gorgona, Vada S, Follonica M, Follonica S, Pioppi, Pantelleria) were not in Hardy-Weinberg equilibrium (Table 4).

In populations that have been sampled at two different depths, frequencies of some alleles were different between the two stands. Moreover, a single allele (*Poc*-26.275) was found to be characteristic of shallow stands, being present only at 5-m depth in Ischia-LA and Milazzo (see also Procaccini & Mazzella 1998). MRPP and permutation of the $(\delta\mu)^2$ distance matrix were also applied to test independence of shallow and deep stands of the same meadow. In five of the seven populations included in the analysis, it was not possible to reject the hypothesis of independence between shallow and deep stand, statistically (Tables 2 and 3). Regarding the other two populations, in Vada the two stands were homogeneous while in the Tavolara population different results were obtained with the two different tests.

Biogeographic trend and gene flow

Gene flow was calculated using two different parameters, θ and ρ . Overall values were significantly higher than zero [$\theta = 0.104$ ($P = 0.037$), $\rho = 0.181$ ($SE = 0.00$)], suggesting the existence of a degree of genetic divergence among meadows. Gene flow estimates were: $Nm^\theta = 2.15$ and $Nm^\rho = 1.16$,

showing moderate gene flow among meadows. Pairwise $(\delta\mu)^2$ distance analysis has been utilized to infer levels of relationships among distinct localities and meadows. MDS plots have been obtained considering both the total number of samples and only one representative of each genotype per meadow, excluding clone-mates (Fig. 3a,b). Both analyses show the existence of two main clusters that represent two distinct groups of meadows. The first is composed of populations sampled north of Civitavecchia, the second, of populations sampled south of Ventotene (see Fig. 1). Relative position of different meadows within the two main clusters is not clearly resolved and mature meadows and seedlings collected in the same localities are not always the most closely grouped.

Discussion

The data presented here were obtained with the same molecular markers utilized in a previous analysis on a limited number of individuals and populations of *Posidonia oceanica* (Procaccini & Mazzella 1998). In the present analysis we confirmed the overall result of low levels of genetic polymorphism of this species in the Western Mediterranean basin. In the 120 individuals previously analysed we found only 14 alleles; five new alleles were detected in the present analysis. However, augmenting the number of samples did not result in a corresponding increase of the per cent number of genotypes. Increasing the number of ramets five-fold (from 120 to 699), in fact, resulted in an almost three-fold decrease of the overall G/N value (from 0.325 to 0.116). In the four localities (Vada, Ventotene, Ischia-LA, Pantelleria) where the number of sampled ramets was almost doubled in respect to the previous analysis, the per cent of distinct genotypes did not change or even decrease (see Fig. 3 in Procaccini & Mazzella 1998 and Table 4 in the present study). This suggests that, with the level of variability detected by the microsatellite markers utilized, the number of ramets sampled in the *P. oceanica* meadows is adequate to give a reliable indication of their genetic polymorphism.

In our analysis of clonality we must take into consideration that, according to P_{gen} values, some of the genotypes can be identical by chance. Nevertheless, considering that P_{gen} values calculated for the six-loci genotypes are biased upward for the presence of a chloroplastic locus (i.e. uniparentally inherited), we are confident that in most cases our estimation of clonality is close to reality.

Studies of population genetics of seagrasses and clonal plants in general have to deal with two main problems, the existence of replicates of the same genotype within the same population (clone-mates) and the identification of the population itself. In long-lived systems, such as *Posidonia oceanica* (Mateo *et al.* 1997), we would expect populations to be the result of a long history of clonal growth and

Table 4 Within population genetic diversity averaged over six polymorphic microsatellite loci in the 20 *Posidonia oceanica* meadows analysed. Observed and expected heterozygosity (H_O and H_E) and Hardy–Weinberg equilibrium (HWE) have been obtained only for the five nuclear loci

	Sample size	No. of genotypes	G/N	D_G	No. of exclusive genotypes	Proportion of polymorphic loci	Mean no. of alleles per locus (SE)	H_O	H_E	HWE
Camogli	35	12	0.34	0.86	0	0.67	1.7 (0.2)	0.117 (0.057)	0.247 (0.113)	*
Meloria M	37	14	0.38	0.93	3	0.67	2.0 (0.4)	0.157 (0.083)	0.234 (0.110)	NS
Meloria St	40	20	0.50	0.95	2	0.67	1.8 (0.3)	0.110 (0.068)	0.290 (0.119)	**
Gorgona	39	14	0.36	0.92	1	0.67	1.8 (0.3)	0.057 (0.042)	0.280 (0.123)	**
Vada M	47	12	0.26	0.87	2	0.67	1.8 (0.3)	0.167 (0.070)	0.238 (0.107)	NS
Vada St	28	18	0.64	0.96	3	0.83	1.8 (0.2)	0.167 (0.061)	0.279 (0.095)	*
Follonica M	38	16	0.42	0.93	0	0.67	1.7 (0.2)	0.112 (0.070)	0.262 (0.110)	**
Follonica St	37	20	0.54	0.94	1	0.67	1.8 (0.3)	0.170 (0.085)	0.294 (0.120)	**
Elba	28	10	0.36	0.85	1	0.67	1.7 (0.2)	0.150 (0.096)	0.260 (0.110)	NS
Civitavecchia	39	13	0.33	0.84	1	0.67	1.7 (0.2)	0.217 (0.097)	0.263 (0.115)	NS
Tonnara	20	8	0.40	0.86	0	0.67	1.7 (0.2)	0.125 (0.079)	0.232 (0.105)	NS
Tavolara	36	9	0.25	0.82	0	0.67	1.7 (0.2)	0.222 (0.099)	0.272 (0.114)	NS
Ventotene	36	14	0.39	0.90	0	0.67	1.7 (0.2)	0.214 (0.093)	0.318 (0.116)	NS
Ischia-LA	40	13	0.33	0.87	7	0.83	1.8 (0.2)	0.340 (0.093)	0.338 (0.080)	NS
Ischia-CDI	37	5	0.16	0.52	0	0.33	1.3 (0.2)	0.167 (0.105)	0.191 (0.119)	NS
Ischia PC†	35	8	0.23	0.82	0	0.67	1.7 (0.2)	0.133 (0.082)	0.233 (0.101)	NS
Ieranto	20	7	0.35	0.82	0	0.50	1.5 (0.2)	0.171 (0.105)	0.277 (0.116)	NS
Pioppi	28	15	0.54	0.94	3	0.67	2.0 (0.4)	0.240 (0.138)	0.321 (0.136)	**
Milazzo	40	14	0.35	0.87	4	0.67	1.8 (0.3)	0.314 (0.121)	0.308 (0.113)	NS
Pantelleria	39	21	0.54	0.95	7	0.83	2.2 (0.3)	0.200 (0.086)	0.363 (0.106)	**

†Seedling patches; NS = not significant; * $P < 0.05$; ** $P < 0.01$.

Fig. 3 MDS (Multidimensional Scaling) analysis performed on the pairwise $(\delta\mu)^2$ distance matrices over the total number of samples (A) and considering only one representative of each genotype per population (B).

sexual reproduction. Previous studies give strong indications for the presence of large clones, in particular in some localities (Procaccini & Mazzella 1998). According to the present data, collecting shoots in *P. oceanica* meadows along the Western coasts of Italy, including part of the Eastern coasts of Corsica and Sardinia, there is only an 11.6% chance of encountering different genotypes. In other words, according to the level of variability detected by our markers, almost 90% of the millions of *Posidonia* shoots present in this geographical area are genetically identical.

Definition of single population units

Actual meadow boundaries can originate from habitat fragmentation and regression of original larger meadows. Can each of the sampled meadows be considered as a discrete population?

To approach this problem we utilized two different statistical tests and the results obtained need to be carefully evaluated. The answer is yes for 14 of the 16 mature meadows that we analysed. In the only two meadows (Gorgona and Follonica) where contrasting results were obtained the observed value of within population Euclidean distance was only slightly higher than the expected average value (Fig. 2). Thus, despite the low number of genotypes found and the fact that many genotypes are present in more

than one meadow, their distribution in single localities is not compatible with a random selection of genotypes from a single population. In other words, spatially disjunct meadows represent genetically distinct groups of individuals and can be considered as distinct populations. Population size is small, as suggested by the low number of genotypes observed. However, the levels of gene flow that we found should be able to prevent complete genetic isolation, with the consequent genetic load and loss of polymorphism (Slatkin 1987).

Within-population genetic diversity and substructuring

The G/N and D_G parameters of genetic diversity calculated here were compared with P_D and D_G parameters calculated on two other monoecious seagrass species, *Zostera marina* and the congeneric *Posidonia australis*, respectively (Waycott 1998; Reusch *et al.* 2000). Values of genetic diversity obtained for the other two species are consistently higher than those obtained in *P. oceanica*. P_D values (comparable with G/N) calculated in *Z. marina* on microsatellite data ranged from 0.33 to 1.0, in 10 different localities, without considering two meadows that were completely uniclinal. In five populations all ramets represented different genets (Reusch *et al.* 2000), in contrast to our study where the more polymorphic population has 64% diverse genotypes. In *P. australis* the D_G value calculated on random amplified polymorphic DNA (RAPD) data, instead, ranged from 0.67 to 1.0, with almost 40% of sampled populations showing values higher than 0.97. This suggests continuous recruitment of diverse new genotypes along the Southern Australian coasts (Waycott 1998). In contrast to these two studies, *P. oceanica* meadows are largely uniclinal, and therefore clonality can be considered as the primary reproductive mode. Sexual reproduction, instead, seems to give a little contribution to increase genetic diversity (Sandmeier *et al.* 1999; Orsini *et al.* 2000). When comparing the genetic polymorphism among seedling patches and their adjacent mature meadows (see Table 3) seedlings were not consistently more diverse. The low genetic diversity observed can result from a number of different factors: (i) high inbreeding, as suggested by homozygosity values (mean $f \approx 0.35$), and including geitonogamy and selfing; (ii) apomictic development of seeds; and (iii) rare sex and seedling's establishment.

The existence of genetic substructuring within meadows was demonstrated statistically in five of the seven populations sampled at two different depths. Previous analysis (Procaccini & Mazzella 1998) had already suggested depth-related genetic distinction in *P. oceanica*, indicated by the presence of one allele segregating in the shallow stand of the Ischia-LA meadow. The same pattern has been observed here in all the new samples considered in this site and was also found in another meadow (Milazzo). Even

greater depth-related genetic substructure was previously found in another seagrass species, *Halophila stipulacea*, analysed by means of RAPD polymerase chain reaction (Procaccini *et al.* 1999). In both species, differences in the ripening period of reproductive structures at different depths always occur, probably related to the temperature and light regime experienced by plants (Procaccini & Mazzella 1998; Procaccini *et al.* 1999). In addition, rate of sexual reproduction and floral initiation cues may differ at depth between season and within-shoot flowering may not occur every year. In the meadows located around the Island of Ischia, for example, during the spring of 2000, fruits were recorded only in stands deeper than 15 m (M.C. Gambi & M.C. Buia, personal communication). All the above considerations allow us to suppose that differences in allele frequencies between depths are due to restricted gene flow more than to adaptation to local habitat conditions.

Biogeographic trend and gene flow

The analysis of gene flow was performed considering both all the genotypes and only one representative of each genotype per population. This was done in order to test the effect of replicate genotypes within the same population on establishing relationships among different localities. The results obtained were comparable, showing the existence of two different population groups, according to geographical location.

Current biogeographic trends in *P. oceanica* can be the result of past history of colonization and persistence in specific areas and actual directions of gene flow, driven by dispersion of pollen, seeds, or vegetative propagules. In particular, the position of seedling patches in the MDS plot reflects only recent dispersion and needs to be carefully considered. Stands of adult plants and of seedlings collected in the same location did not always segregate together in the MDS plot. Recruitment seems to be autochthonous only in the Vada population, while seedlings collected in the other locations could come from any of the populations of the same geographical area. Although persistence on the surface and in the water column, and consequent dispersal distance of pollen and seeds, is not well known in *P. oceanica*, our data suggest that it is effective at least within each of the two geographical areas considered. Distinction between southern and northern populations, instead, may be related to current regimes in the whole area. Analysing direction and intensity of the dominant surface currents along the eastern Italian coasts we observe two main patterns (Tait 1984): (i) seasonal local circulation cells in the North, centre and South Tyrrhenian Sea, that could determine isolation of different parts of the whole system; and (ii) a persistent South–North current along the coast that could homogenize genetic characteristics of populations. Nevertheless, it is important to consider that local circulation

cells are seasonal and are present in spring–summer, when pollen (in early spring) and seeds (in early summer) are released (Buia & Mazzella 1991). More studies with appropriate sampling design are needed taking into consideration current speed and dispersal capability of *P. oceanica*.

In conclusion, our analysis showed the existence of clear patterns of genetic structure in *P. oceanica* in the area considered in the analysis. *P. oceanica*, in fact, is present in separate meadows that represent discrete populations, characterized by low genetic diversity and small population size. Comparable levels of genetic variability between mature meadows and seedlings were found. Patterns of genetic relatedness among populations seem to be in accord with direction of dominant current flux in the whole area, separating South Tyrrhenian from North Tyrrhenian populations. Seasonal circulation cells, instead, seem to support moderate gene flow within the single areas. Genetic distinction of single populations and genetic substructure within populations, together with the finding of the limited role of sexual reproduction in increasing genetic variability, should be causes for concern for the persistence of this essential resource in the Mediterranean basin.

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The results of this work come from a multidisciplinary approach to the study of Mediterranean seagrass ecosystems, carried out at the Benthic Ecology Laboratory of the Stazione Zoologica 'A.Dohrn'. This work is one of a series of papers which explore population structure and phylogeography of *Posidonia oceanica*. L.O. and M.V.R. have worked for this study under the direction of G.P., who is interested in population genetics and phylogeography of plant and algal systems in the Mediterranean sea, with special emphasis on the *Posidonia* ecosystem. M.S. provided support for data analysis and developed *ad hoc* matrix permutation tests.