Journal of Biogeography (J. Biogeogr.) (2007) 34, 963–976

# Vicariance patterns in the Mediterranean Sea: east–west cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*

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## ABSTRACT

**Aim** The seagrass, *Posidonia oceanica* is a clonal angiosperm endemic to the Mediterranean Sea. Previous studies have suggested that clonal growth is far greater than sexual recruitment and thus leads to low clonal diversity within meadows. However, recently developed microsatellite markers indicate that there are many different genotypes, and therefore many distinct clones present. The low resolution of markers used in the past limited our ability to estimate clonality and assess the individual level. New high-resolution dinucleotide microsatellites now allow genetically distinct individuals to be identified, enabling more reliable estimation of population genetic parameters across the Mediterranean Basin. We investigated the biogeography and dispersal of *P. oceanica* at various spatial scales in order to assess the influence of different evolutionary factors shaping the distribution of genetic diversity in this species.

**Location** The Mediterranean.

**Methods** We used seven hypervariable microsatellite markers, in addition to the five previously existing markers, to describe the spatial distribution of genetic variability in 34 meadows spread throughout the Mediterranean, on the basis of an average of 35.6 (± 6.3) ramets sampled.

**Results** At the scale of the Mediterranean Sea as a whole, a strong east–west cleavage was detected (AMOVA). These results are in line with those obtained using previous markers. The new results showed the presence of a putative secondary contact zone at the Siculo-Tunisian Strait, which exhibited high allelic richness and shared alleles absent from the eastern and western basins. *F* statistics (pairwise $\theta$ ranges between 0.09 and 0.71) revealed high genetic structure between meadows, both at a small scale (about 2 to 200 km) and at a medium scale within the eastern and western basins, independent of geographical distance. At the intrameadow scale, significant spatial autocorrelation in six out of 15 locations revealed that dispersal can be restricted to the scale of a few metres.

**Main conclusions** A stochastic pattern of effective migration due to low population size, turnover and seed survival is the most likely explanation for this pattern of highly restricted gene flow, despite the importance of an a priori seed dispersal potential. The east–west cleavage probably represents the outline of vicariance caused by the last Pleistocene ice age and maintained to this day by low gene flow. These results emphasize the diversity of evolutionary processes shaping the genetic structure at different spatial scales.

**Keywords**

**Clonal plant, contact zone, genetic divergence, glaciations, Mediterranean biogeography, microsatellites, Pleistocene, *Posidonia oceanica* speciation.**

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## INTRODUCTION

Large-scale panmixis is expected in the sea and has often been observed there, being primarily attributed to the scarcity of strong dispersal barriers (Vermeij, 1987). However, this a priori expectation may only reflect our limited understanding of genetic boundaries in the marine environment, rather than any objective evidence (Crosetti *et al.*, 1994; Hedgecock, 1994; Palumbi, 1994). An increasing number of studies report restricted gene flow between marine populations, despite high dispersal potential in some cases (e.g. Hedgecock, 1986; Palumbi *et al.*, 1997; Planes & Fauvelot, 2002; Arnaud-Haond *et al.*, 2003; Billot *et al.*, 2003; Warner & Palumbi, 2003).

Vicariance due to Pleistocene glacial episodes, and the resulting variations in sea level and surface temperature, often emerges as the mechanism generating genetic differentiation in marine invertebrates (Palumbi *et al.*, 1997; Duke *et al.*, 1998), fish (Planes & Fauvelot, 2002; Fauvelot *et al.*, 2003) and mammals (Hare *et al.*, 2002). These events help explain genetic patterns in the Mediterranean biota, where the Siculo-Tunisian Strait is as an important genetic boundary for several species (Borsa, 1997; Bahri-Sfar *et al.*, 2000; Nikula & Vainola, 2003), including an east–west cleavage for sea bass (*Dicentrarchus labrax*; Bahri-Sfar *et al.*, 2000), bivalves (*Mytilus galloprovincialis* and *Cerastoderma glaucum*; Quesada *et al.*, 1995; Nikula & Vainola, 2003) and several fish species (Borsa, 1997).

The marine angiosperm *Posidonia oceanica* is endemic to the Mediterranean Sea (Den Hartog, 1970), where its meadows are more or less continuously distributed along the coasts from southern Spain and northern Morocco to the eastern Levantine Sea, and represent the most productive coastal ecosystems. This species provides a good model for exploring patterns of genetic structure at different scales across the Mediterranean Sea and elucidating which factors could be influencing present-day patterns. The first molecular studies, using allozymes (Capiomont *et al.*, 1996), M13-DNA fingerprinting (Procaccini *et al.*, 1996) and random amplification of polymorphic DNA (Procaccini & Mazzella, 1996), showed such low levels of genotypic diversity that it was not possible to assess population genetic structure. Further studies with trinucleotide microsatellites (Procaccini & Waycott, 1998; Procaccini *et al.*, 2001) revealed a slightly higher level of polymorphism, although it was not sufficient to recognize distinct individuals on the basis of their multilocus genotypes. The apparent heterogeneity in the distribution of genetic variability was mainly attributed to the predominance of clonal growth, resulting in a very low effective population size (Procaccini *et al.*, 2001, 2002). The pattern of genetic divergence at the scale of the Mediterranean Sea showed an east–west cleavage (Procaccini *et al.*, 2001, 2002), consistent with the reported phenological differences between basins (Bussoti *et al.*, 1998). Karyotypic heterogeneity was also observed among localities in the Mediterranean, with distinct karyotypes detected in one meadow in northern Africa (in Algeria, along the coast near Algiers; Semroud *et al.*, 1992). These differences led some authors (Semroud *et al.*, 1992; Bussoti *et al.*, 1998) to question the taxonomic status of *P. oceanica* across the Mediterranean Sea. However, further investigation of the evolutionary factors shaping genetic structures at different spatial scales and screening for possible reproductive isolation within the Mediterranean Sea required the clear identification of genetic individuals with higher-resolution markers.

The recent development of more efficient polymorphic microsatellite markers (Alberto *et al.*, 2003) has provided new opportunities to unravel the genetic structure of *P. oceanica* meadows. Analyses with these new microsatellites performed on samples from eight meadows revealed that the earlier conclusion that populations were largely clonal was an artefact due to the low power of previous markers, and that clonal diversity in the meadows is much higher than previously believed (Arnaud-Haond *et al.*, 2005). The availability of new, powerful markers has now made it possible to detect higher allelic richness and to distinguish individuals in a sample of shoots on the basis of their multilocus genotype. This means that reliable estimates can now be made of genetic variability and structure at different spatial scales in the Mediterranean Sea (Arnaud-Haond *et al.*, 2005).

In the present study, we use an optimized set of seven dinucleotide microsatellite markers (Arnaud-Haond *et al.*, 2005), together with the established trinucleotide microsatellites (four trinucleotide and one heptanucleotide microsatellite; Procaccini & Waycott, 1998), to analyse samples from 34 *P. oceanica* meadows distributed across the Mediterranean from Spain to Cyprus. Our objectives were: (1) to screen for footprints of historical events that have shaped the current distribution of genetic diversity, and (2) to identify the present-day factors that maintain this structure at different spatial scales. The first goal was achieved by analysing samples collected throughout the Mediterranean Sea and by screening the genetic composition of the intermediate geographical zone between western and eastern basins and the Siculo-Tunisian Strait to search for patterns typical of either a hybrid or a secondary contact zone. The second goal was addressed by examining the genetic structure among meadows at regional (intrabasin) and local scales. Autocorrelation analysis was used to test for the occurrence of limited dispersal at the intrameadow scale.

## MATERIALS AND METHODS

### Sampling

Approximately 40 *P. oceanica* shoots were sampled in each of 34 localities, ranging from the Spanish Mediterranean coast to Cyprus (Fig. 1 & Table 1), and spanning between 10 and about 3500 km. Shoots were collected at randomly drawn coordinates across a $20 \times 80$ m area in 16 meadows (Fig. 1 & Table 1, with sampling code a), except in one of the meadows


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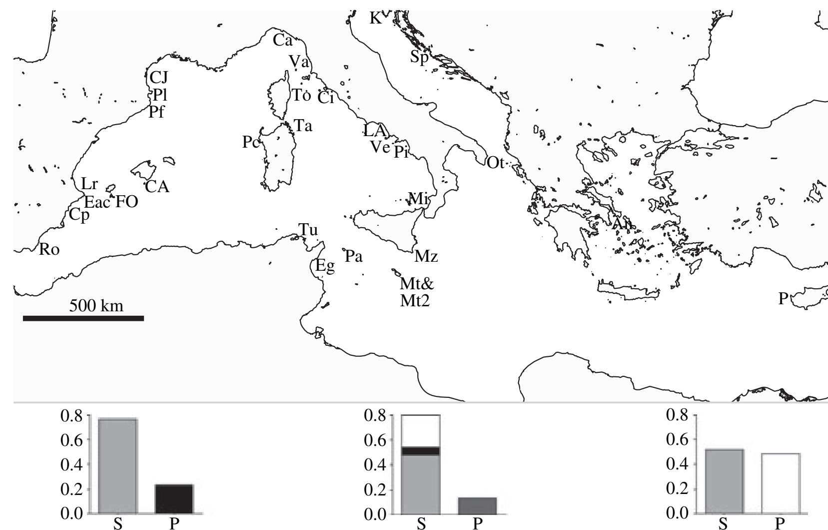


**Figure 1** Map of the sampling locations where *P. oceanica* was collected. Abbreviations for locality names are detailed in Table 1, except for the Balearic Islands for which only the island was indicated: Formentera (FO) and Cabrera (CA). The proportion of shared (S) and private (P) alleles is detailed in western, eastern, and intermediate groups. For the shared alleles within the intermediate group (group limits are indicated by dotted lines), the detail of the proportion of alleles shared among all groups (striped bar chart), with western (white) and with eastern (black) groups is provided.

from Formentera (Balearics) where, due to an incident during a collection dive, some coordinates could not be recorded. For the remaining localities, a shoot was collected every 7–8 m (Table 1, with sampling code b), following a linear transect, as detailed in Procaccini *et al.* (2001) and only analysed with the original set of tri- and heptanucleotide microsatellites (Procaccini & Waycott, 1998). A meristematic portion of each shoot was removed, desiccated and preserved in silica gel.

As shown in Fig. 1, the western basin was represented by samples from Spain, France and western Italy to northern Sicily (Ro, Cp, Eac, Lr, Pf, CJ, Pl, Fec, Fi, Fep, Cec, Csm, Cs7, To, Pc, Ca, Ta, Va, Ci, Ve, Pi, LA, Mi; 'western group'); the centre of the Mediterranean Sea, specifically the region of the Siculo-Tunisian Strait separating Tunisia from southern Sicily, was represented by samples from southern Sicily, Tunisia and Malta (Pa, Mz, Tu, Eg, Mt, Mt2; 'central group'); and the eastern basin was represented by samples from eastern Italy, Croatia, Slovenia, Greece and Cyprus (Ot, Sp, K, An, P; 'eastern group').

## Microsatellite analysis

Genomic DNA was isolated following a standard CTAB extraction procedure (Doyle & Doyle, 1987) for the 15 new populations. For the remaining populations, the extraction procedure is detailed in Procaccini *et al.* (2001, 2002). Each shoot or connected shoots sampled together will be referred to as a 'sampling unit' (SU), or ramet in this work.

All 34 meadows (Table 1) were analysed with the most efficient combination (Arnaud-Haond *et al.*, 2005) of seven dinucleotide nuclear markers (Alberto *et al.*, 2003), Po4-3, Po5, Po5-10, Po5-39, Po5-40, Po5-49 and Po 15. These data were combined with data from five nuclear tri- and heptanucleotide microsatellites: *Poc*-5, *Poc*-26, *Poc*-35, *Poc*-42 and *Poc*-45 (Procaccini & Waycott, 1998), generated in this study for the 15 newly sampled meadows (meadows with sampling scheme a in Table 1) and already available for the remaining 19 meadows (Procaccini *et al.*, 2002; meadows with sampling scheme b in Table 1).

## Clone discrimination

We used the round-robin method (Parks & Werth, 1993) to estimate the allelic frequencies at nuclear loci in each population. This subsampling approach avoids overestimation of rare allelic frequencies by estimating the allelic frequencies for each locus on the basis of a sample pool composed of all the genotypes distinguished on the basis of all loci, except the one for which the allelic frequencies were estimated. This procedure was repeated for all loci and the unique genotype probability, taking into account Wright's (1951) inbreeding coefficient estimated for each locus after the exclusion of identical multilocus genotypes (Young *et al.*, 2002). This was calculated as:

$$p_{\mathrm{gen(f)}} = \prod_{i=1}^{l} \{(f_i g_i) \times [1 + (z_i \times (F_{\mathrm{is}(i)}))]\} 2^h$$

with $l$ representing the number of loci, $h$ the number of heterozygous loci and $f$ and $g$ the allelic frequencies of the alleles $f$ and $g$ at the $i$ th locus (with $f$ and $g$ identical for homozygotes, $F_{\mathrm{is(i)}}$ the $F_{\mathrm{is}}$ estimated for the $i$ th locus with the round-robin method, and $z_{\mathrm{i}} = 1$ for the $i$ th locus if it is homozygous and $z_{\mathrm{i}} = -1$ for the $i$ th locus if it is heterozygous). When the same genotype is detected more than once ($n$) in a population sample composed of $N$ ramets, the probability that the samples actually originate from distinct reproductive events (i.e. from separate genets) is described by the binomial expression (Tibayrenc *et al.*, 1990; Parks & Werth, 1993):

$$p_{\mathrm{sex}} = \sum_{i=n}^{N} \frac{N!}{i!(N-i)!} \left[p_{\mathrm{gen(f)}}\right]^i \left[1 - p_{\mathrm{gen(f)}}\right]^{N-i}$$

where $n$ is the number of sampled ramets with the same multilocus genotype, $N$ is the sample size and $p_{\mathrm{gen}}(\mathrm{f})$ is the


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**Table 1** Sampling details. Sampling locations, sampling code (island code), approximate GPS coordinates, sampling scheme (S; a are samples collected according to random coordinates in a 80 × 20 m area; b are samples collected each 7–8 m along a linear transect), approximate depth in metres ($D$), sample size ($N_s$).

| | Sampling localities | Code | GPS coordinates | S | $D$ | $N_s$ |
|---|---|---|---|---|---|---|
| Spain (peninsula) | Rodalquilar | Ro | 2°00.53′ W, 36°51.21′ N | a | 4 | 40 |
| | Campomanes | Cp | 0°0.57′ E, 38°37.54′ N | a | 7 | 31 |
| | El Arenal-Calpe | Eac | 0°3.06′ E, 38°38.37′ N | a | 6 | 39 |
| | Las Rotes | Lr | 0°8.56′ E, 38°50.03′ N | a | 6 | 40 |
| | Punta de Fanals | Pf | 2°50.56′ E, 41°41.58′ N | a | 17 | 38 |
| | Cala Jonquet | CJ | 3°17.36′ E, 42°18.19′ N | a | 6 | 39 |
| | Port lligat | Pl | 3°17.58′ E, 42°17.61′ N | a | 8 | 40 |
| Spain (Balearic Islands) | Es Caló des Oli (Formentera) | Fec (FO) | 1°24.16′ E, 38°43.49′ N | a | 5 | 40 |
| | Illetas (Formentera) | Fi (FO) | 1°25.83′ E, 38°45.37′ N | a | 7 | 35 |
| | Es Pujols (Formentera) | Fep (FO) | 1°27.27′ E, 38°43.74′ N | a | 5 | 40 |
| | Es Castell (Cabrera) | Cec (CA) | 2°55.83′ E, 39°9.16′ N | a | 5 | 40 |
| | Sta María 13 m (Cabrera) | Csm (CA) | 2°56.92′ E, 39°9.07′ N | a | 13 | 35 |
| | Sta María 7 m (Cabrera) | Cs7 (CA) | 2°56.96′ E, 39°9.00′ N | a | 7 | 40 |
| France (Corsica) | Tonnara | To | 9°06.00′ E, 41°25.00′ N | b | < 10 | 20 |
| Italy | Porto Conte (Sardegna) | Pc | 8°12.00′ E, 40°36.00′ N | b | < 10 | 20 |
| | Camogli | Ca | 9°09.00′ E, 44°20.00′ N | b | < 10 | 40 |
| | Tavolara (Sardegna) | Ta | 9°41.00′ E, 40°53.00′ N | b | 5–20 | 40 |
| | Vada | Va | 10°22.00′ E, 43°18.00′ N | b | 5–20 | 29 |
| | Civitavecchia | Ci | 11°45.00′ E, 42°07.00′ N | b | < 10 | 40 |
| | Ventotene | Ve | 13°25.00′ E, 40°47.00′ N | b | < 10 | 38 |
| | Pioppi | Pi | 15°04.00′ E, 40°10.00′ N | b | < 10 | 28 |
| | Lacco Ameno | LA | 13°53.00′ E, 40°45.00′ N | b | 5–20 | 43 |
| | Milazzo | Mi | 15°13.00′ E, 38°13.00′ N | b | < 10 | 40 |
| | Pantelleria Island | Pa | 11°58.00′ E, 36°45.00′ N | b | 5–20 | 37 |
| | Marzamemi (Sicily) | Mz | 15°00.49′ E, 36°43.29′ N | a | 8 | 38 |
| | Otranto | Ot | 18°28.00′ E, 40°10.00′ N | b | < 10 | 29 |
| Tunisia | Tunis | Tu | 10°19.00′ E, 36°46.00′ N | b | < 10 | 40 |
| | Ergla | Eg | 10°36.00′ E, 35°53.00′ N | b | < 10 | 25 |
| Malta | Malta 1 | Mt | 14°33.00′ E, 35°51.00′ N | b | 5–20 | 39 |
| | Malta 2 | Mt2 | 14°20.00′ E, 35°59.00′ N | b | 25 | 38 |
| Croatia | Split | Sp | 16°39.00′ E, 43°15.00′ N | b | < 10 | 22 |
| Slovenia | Koper | K | 13°42.00′ E, 45°33.00′ N | b | 3 | 36 |
| Greece | Agios Nicolaos | An | 23°55.62′ E, 37°42.97′ N | a | 6 | 40 |
| Cyprus | Paphos | P | 32°26.23′ E, 34°43.54′ N | a | 10 | 38 |

probability of the common genotype. Detection of individuality in clonal plants can be biased by somatic mutations (Klekowski, 1997, 2003), particularly when individuals are long-lived, as in the case of *P. oceanica* where clones date back millennia (Marbà & Duarte, 1998; Hemminga & Duarte, 2000). Furthermore, increasing the number of loci used increases the likelihood of scoring errors (Douhovnikoff & Dodd, 2003; Meirmans & Van Tienderen, 2004). Therefore, we further investigated the data set by screening, within each locality, all pairs of ramets that were distinct for two loci or fewer among the 13 single locus genotypes. For all pairs, the distinct loci were removed and the probability, $p_{sex}$, was re-estimated on the basis of the remaining identical ones. A probability lower than 0.01 led to rejection of the null hypothesis that those ramets belonged to individuals derived from distinct sexual events. The discrepancy was then probably due to somatic mutations or scoring errors and the ramets were considered as belonging to the same genetic individual (or genets) corresponding to a multilocus lineage (MLL), to which the slightly distinct, multilocus genotypes were considered to belong.

Clonal diversity was estimated as:

$$R = \frac{G-1}{N-1}$$

with $G$ representing the number of multilocus genotypes or multilocus lineages (when taking into account possible somatic mutations or scoring errors) discriminated in the sample and $N$ representing the number of sampled ramets. Calculations were performed using software (GenClone) written for this purpose (Arnaud-Haond & Belkhir, 2007).

### Genetic data analysis

After the identification of ramets belonging to the same genets, replicates were removed from the data set to perform the


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following calculations using the GENETIX 4.0 package (Belkhir *et al.*, 1996–2001). When slightly distinct genotypes were determined as belonging to the same MLL (genets), the most common genotype was used. Genetic diversity within populations was estimated by unbiased ($H_{nb}$) and observed ($H_{obs}$) gene diversity (Nei, 1987). We used a permutation procedure (1000 permutations) to test whether a particular estimate of the overall inbreeding coefficient ($F_{is}$) was significantly different from 0. The two-locus correlation coefficient $R^2$ (Weir, 1979) was estimated as described by Black & Krafsur (1985), and its significance (i.e. departure from zero) was tested by a permutation approach.

Allelic richness was estimated in all population samples, both on the basis of the entire sample, and for comparative purposes by resampling $1000 \times 20$ ramets (the smallest sampling size in Tonnara and Porto Conte) and then using the average richness (± SE) over those 1000 replicated subsamples. Allelic richness in a large region may be high either because one population has more alleles or because different populations have different alleles even though intrapopulation diversity might be low, although this is not commonly tested for. Therefore, in order to compare the diversity in western, eastern and the central regions without having this estimate influenced by possible variations in clonal diversity between regions, all distinct genets identified in each region were pooled into three groups, and a subsampling approach (subsampling $1000 \times 50$ genets) was used to obtain standardized, and therefore comparable, estimates of allelic richness in each of the three regions. The confidence interval obtained for each three groups allowed us to test the null hypothesis of equal allelic richness in all three groups against the alternative hypothesis of higher allelic richness in the central region.

The hierarchical structure of genetic variation at the whole Mediterranean scale was examined using ARLEQUIN software (Excoffier *et al.*, 2005). Components of genetic variance were computed at two hierarchical levels: between the east and west Mediterranean ($\Phi_{CT}$), and among geographical locations within those two groups ($\Phi_{SC}$). Without any a priori hypothesis about the central group (southern Sicily, Tunisia and Malta) being genetically closer to eastern or western groups, two series of hierarchical analyses were performed grouping central populations with eastern and western populations, respectively, in order to assess which cluster would maximize the proportion of total genetic variance due to differences between groups of populations.

In order to illustrate the spatial variation in the genetic composition of populations from different geographical areas, correspondence analysis (Benzécri, 1982) was performed using GENETIX 4.01 (Belkhir *et al.*, 1996–2001) on the matrix of allelic frequencies per sample. This representation can give an easily interpreted picture of the partition of genetic variability among populations, and Guinand (1996) showed that the eigenvalues of each axis are analogous to partial $F_{st}$.

Genetic differentiation ($F_{st}$) was estimated between pairs of populations with the estimator $\theta$ (Weir & Cockerham, 1984), and its significance was tested using 1000 random permutations of the individuals between samples. The relationship between genetic and geographical distances was screened using a Mantel test at three geographical scales: at the Mediterranean scale between all sample pairs, at the regional scale between samples within eastern and western groups, and at the local scale among localities within the Balearic Island (less than 200 km). As suggested by Rousset (1997) in a two-dimensional model, we plotted $[\hat{\theta}]$ against the logarithm of the geographical distance in kilometres for each population pair. In order to avoid bias in the test by over-representing some distance ranges, sample pairs less than 20 km apart had only one locality included in the analysis. These analyses and tests were performed using the GENETIX 4.0 package (Belkhir *et al.*, 1996–2001).

Restriction to gene flow at the intrameadow scale was tested by performing spatial autocorrelation analysis in 15 populations for which all sampling coordinates were available (Table 1). The methods described by Vekemans & Hardy (2004) were used to estimate the statistic *Sp* of the regression between the logarithm of geographical distance and the co-ancestry coefficient defined by Ritland (1996). This relatedness coefficient has been proven to be more powerful than others in most cases (Vekemans & Hardy, 2004), particularly when using hypervariable markers. The autocorrelation analyses were conducted twice for each sample: (1) first, including all ramets, which principally estimates the neighbourhood of ramets of the same genet, and (2) resampling 1000 data sets in which only one ramet belonging to each genet was randomly included. Due to the use of random coordinates and the variable number of genets available in each sample, we provided the program with a number of classes (6), among which the total number of individual pairs was evenly distributed. These calculations were also performed using GenClone (Arnaud-Haond & Belkhir, 2007).

## RESULTS

### Clonal diversity

The clonal diversity revealed by the 12 loci analysed (Table 2) ranged between 0.00 (Split) and 1.00 (Vada). Among the 875 distinct multilocus genotypes (MLG) distinguished, about 10% (85) had a slightly distinct MLG and were found to have a significant probability of not being separated from each other by a sexual event. They were grouped with similar MLG to MLL. Among the 790 defined MLLs, 763 had all replicates exhibiting a significant probability of less than 0.01 of belonging to individuals issued from two distinct events of sexual reproduction (i.e. distinct genets). All samples belonging to identical MLL were then considered as samples derived from different ramets of the same genetic individual or genet (i.e. originated from a single seed), and replicates were removed from the data set for further genetic analyses. In the case of the remaining 27 multiple MLLs (less than 4%,


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**Table 2** Sampling locations, number of samples genotyped ($N_s$), number of distinct genets (MLLs) identified, clonal diversity and total number of alleles on the entire sample (MLL, $R$ and $A$, respectively) and the average (± SE) of 1000 subsamples of 20 ramets ($MLL_{20}$, $R_{20}$ and $A_{20}$); unbiased ($H_{nb}$) and observed heterozygosity ($H_{obs}$), deviation from Hardy–Weinberg equilibrium ($F_{is}$, *significant after a 1000 permutation test) with the 12 nuclear microsatellites.

| Sampling localities | $N_s$ | MLL | $MLL_{20}$ | $R$ | $R_{20}$ | $A$ | $A_{20}$ | $H_{nb}$ | $H_{obs}$ | $F_{is}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Rodalquilar | 40 | 22 | 13.77 ± 0.05 | 0.54 | 0.67 ± 0.20 | 35 | 32.60 ± 0.04 | 0.38 | 0.25 | −0.08 |
| Campomanes | 31 | 24 | 16.20 ± 0.04 | 0.77 | 0.80 ± 0.00 | 47 | 41.62 ± 0.13 | 0.45 | 0.49 | −0.10* |
| El Arenal-Calpe | 39 | 33 | 17.86 ± 0.04 | 0.84 | 0.89 ± 0.00 | 40 | 35.83 ± 0.07 | 0.44 | 0.47 | −0.07 |
| Las Rotes | 40 | 27 | 15.18 ± 0.05 | 0.67 | 0.75 ± 0.00 | 38 | 33.77 ± 0.08 | 0.42 | 0.42 | −0.01 |
| Punta de Fanals | 38 | 26 | 14.75 ± 0.05 | 0.68 | 0.72 ± 0.00 | 32 | 29.07 ± 0.04 | 0.29 | 0.36 | −0.34** |
| Cala Jonquet | 39 | 25 | 14.67 ± 0.05 | 0.63 | 0.72 ± 0.00 | 37 | 33.00 ± 0.06 | 0.36 | 0.39 | −0.10* |
| Port lligat | 40 | 13 | 7.89 ± 0.04 | 0.31 | 0.36 ± 0.00 | 34 | 29.62 ± 0.06 | 0.40 | 0.58 | −0.47** |
| Es Caló des Oli | 40 | 16 | 9.96 ± 0.05 | 0.38 | 0.47 ± 0.00 | 45 | 39.36 ± 0.09 | 0.49 | 0.40 | 0.18** |
| Illetas | 35 | 22 | 13.05 ± 0.05 | 0.62 | 0.63 ± 0.00 | 38 | 34.62 ± 0.05 | 0.40 | 0.47 | −0.15* |
| Es Pujols | 40 | 30 | 16.79 ± 0.04 | 0.74 | 0.83 ± 0.00 | 38 | 33.34 ± 0.05 | 0.36 | 0.40 | −0.14* |
| Es Castel | 40 | 7 | 5.40 ± 0.02 | 0.15 | 0.23 ± 0.00 | 26 | 23.87 ± 0.06 | 0.26 | 0.38 | −0.48** |
| Sta María 13 | 35 | 22 | 14.56 ± 0.04 | 0.62 | 0.71 ± 0.00 | 30 | 29.02 ± 0.03 | 0.39 | 0.43 | −0.14* |
| Sta María 7 | 40 | 24 | 14.37 ± 0.05 | 0.59 | 0.70 ± 0.00 | 34 | 30.01 ± 0.06 | 0.28 | 0.32 | −0.15* |
| Tonnara | 20 | 17 | 17.00 ± 0.00 | 0.84 | 0.84 ± 0.00 | 27 | 27.00 ± 0.00 | 0.37 | 0.35 | 0.04 |
| Porto Conte | 20 | 19 | 19.00 ± 0.00 | 0.95 | 0.95 ± 0.00 | 27 | 27.00 ± 0.00 | 0.37 | 0.45 | −0.24** |
| Camogli | 40 | 37 | 19.23 ± 0.02 | 0.92 | 0.96 ± 0.00 | 38 | 36.95 ± 0.03 | 0.44 | 0.37 | 0.09** |
| Tavolara | 40 | 20 | 12.04 ± 0.05 | 0.49 | 0.58 ± 0.00 | 35 | 33.91 ± 0.03 | 0.44 | 0.47 | −0.08 |
| Vada | 29 | 29 | 20 ± 0.00 | 1 | 1.00 ± 0.00 | 43 | 40.44 ± 0.04 | 0.46 | 0.42 | 0.09 |
| Civitavecchia | 40 | 28 | 15.33 ± 0.05 | 0.69 | 0.75 ± 0.00 | 38 | 34.20 ± 0.05 | 0.46 | 0.49 | −0.07 |
| Ventotene | 38 | 33 | 18.46 ± 0.03 | 0.86 | 0.92 ± 0.00 | 47 | 42.55 ± 0.05 | 0.47 | 0.50 | −0.05 |
| Pioppi | 28 | 25 | 18.32 ± 0.03 | 0.89 | 0.91 ± 0.00 | 50 | 47.73 ± 0.04 | 0.50 | 0.48 | 0.03 |
| Lacco Ameno | 43 | 33 | 17.25 ± 0.04 | 0.76 | 0.85 ± 0.00 | 38 | 35.19 ± 0.04 | 0.42 | 0.48 | −0.15** |
| Milazzo | 40 | 19 | 12.00 ± 0.05 | 0.46 | 0.58 ± 0.00 | 41 | 37.64 ± 0.06 | 0.44 | 0.50 | −0.16** |
| Tunis | 40 | 34 | 18.53 ± 0.03 | 0.85 | 0.92 ± 0.00 | 67 | 55.68 ± 0.12 | 0.49 | 0.54 | −0.12** |
| Ergla | 25 | 9 | 8.14 ± 0.02 | 0.33 | 0.38 ± 0.00 | 30 | 29.56 ± 0.02 | 0.26 | 0.32 | 0.06 |
| Pantelleria | 37 | 35 | 19.44 ± 0.02 | 0.95 | 0.97 ± 0.00 | 58 | 52.22 ± 0.06 | 0.53 | 0.57 | −0.07 |
| Marzamemi | 38 | 32 | 18.07 ± 0.03 | 0.84 | 0.90 ± 0.00 | 59 | 49.60 ± 0.09 | 0.51 | 0.47 | 0.08 |
| Malta 1 | 39 | 29 | 16.52 ± 0.04 | 0.74 | 0.82 ± 0.00 | 55 | 48.08 ± 0.08 | 0.43 | 0.44 | −0.02 |
| Malta2 | 38 | 19 | 12.08 ± 0.05 | 0.49 | 0.58 ± 0.00 | 29 | 26.35 ± 0.06 | 0.34 | 0.37 | −0.10** |
| Otranto | 29 | 23 | 16.47 ± 0.03 | 0.79 | 0.81 ± 0.00 | 39 | 36.14 ± 0.06 | 0.30 | 0.35 | −0.19** |
| Split | 22 | 1 | 1 ± 0.00 | 0 | 0.00 ± 0.00 | 18 | 18.00 ± 0.00 | 0.50 | 0.50 | – |
| Koper | 36 | 1 | 1 ± 0.00 | 0 | 0.00 ± 0.00 | 15 | 15.00 ± 0.00 | 0.13 | 0.25 | – |
| Agios Nicolaos | 40 | 29 | 15.98 ± 0.05 | 0.72 | 0.79 ± 0.00 | 44 | 38.74 ± 0.06 | 0.40 | 0.32 | 0.20** |
| Paphos | 38 | 26 | 15.12 ± 0.04 | 0.68 | 0.74 ± 0.00 | 40 | 35.70 ± 0.05 | 0.42 | 0.49 | −0.17** |

distributed in 13 populations), from which replicates could not all be confidently removed, $n$ replicates of the same MLL (considered as distinct genets derived from $n$ distinct seeds) were kept for further analysis, and $n + 1$ corresponded to the number of distinct reproductive events for which $p$ was less than 0.01.

## Genetic diversity, Hardy–Weinberg equilibrium, linkage disequilibrium

The total number of alleles (Table 2) found varied between 15 (Koper) and 67 (Tunis) and the average number of alleles in subsamples of 20 ramets ranged between 15 (Koper) and 57 (Tunis). In order to compare allelic richness, the two samples from the Adriatic Sea (Split and Koper) that exhibited only one genotype were treated separately (their average was 16.5 alleles) from the remaining eastern samples (36 alleles). Using these standardized values, lower allelic richness was observed in eastern (36) and western (34) populations compared with the central populations (44). Finally, the global standardized allelic richness estimated by subsampling (1000 subsamples of 50 genets) among the genets in eastern (95% CI [54, 63]), western (95% CI [58, 71]) and central (95% CI [75, 89]) groups demonstrated a significantly higher level of genetic diversity in the putative contact zone than in the eastern and western basins.

The unbiased heterozygosity $H_{nb}$ ranged from 0.13 (Koper) to 0.53 (Pantelleria). The observed heterozygosity $H_{obs}$ was significantly different from $H_{nb}$ in 18 samples where heterozygote excess was detected (Table 2), and in the sample from Agios Nicolaos and Camogli, where significant heterozygote deficiency was observed. Repeated heterozygote excess, together with a high variance of $F_{is}$ among loci were also observed in this data set (data not shown). These characteristics are common in


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**Table 3** Hierarchical analysis of molecular variance, based on 12 microsatellite markers analysed on samples of wild populations of *P. oceanica* distributed among two pre-defined groups corresponding to the west (Ro, Cp, Eac, Lr, Pf, CJ, Pl, Fec, Fi, Fep, Cec, Csm, Cs7, To, Pc, Ca, Ta, Va, Ci, Ve, Pi, LA, Mi, Pa, Mz, Tu, Eg, Mt, Mt2) and east (Ot, Sp, K, An, P) Mediterranean. The definition of each statistic is given in the Methods section. *P* values were based on 1000 random permutations.

| | Between E–W groups | | | Among populations within groups | | | Among individuals within populations | | |
|---|---|---|---|---|---|---|---|---|---|
| Degrees of freedom | 1 | | | 32 | | | 1830 | | |
| Locus | SSD | % | $\Phi_{CT}$ | SSD | % | $\Phi_{SC}$ | SSD | % | $\Phi_{ST}$ |
| Po15 | 7.20 | 1.20 | 0.01 | 147.91 | 19.20 | 0.19** | 596.62 | 79.60 | 0.20** |
| Po5 | 92.68 | 38.78 | 0.39** | 121.57 | 13.60 | 0.22** | 418.70 | 47.62 | 0.52** |
| Po5-40 | 20.08 | 6.60 | 0.07** | 147.16 | 15.93 | 0.17** | 687.73 | 77.47 | 0.23** |
| Po5-49 | 24.05 | 10.91 | 0.11** | 148.34 | 21.76 | 0.24** | 454.52 | 67.32 | 0.33** |
| Po5-10 | 51.75 | 22.21 | 0.22** | 184.62 | 23.44 | 0.30** | 429.69 | 54.35 | 0.46** |
| Po4-3 | 13.55 | 6.74 | 0.07 | 126.51 | 23.47 | 0.25** | 373.42 | 69.79 | 0.30** |
| Po5-39 | 3.94 | 1.04 | 0.01 | 83.21 | 17.97 | 0.18** | 362.70 | 81.00 | 0.19** |
| *Poc*-35 | 18.35 | 9.26 | 0.09* | 151.20 | 27.36 | 0.30** | 351.75 | 63.38 | 0.37** |
| *Poc*-45 | 45.83 | 26.41 | 0.26** | 121.68 | 19.94 | 0.27** | 326.62 | 53.65 | 0.46** |
| *Poc*-42 | 74.71 | 41.78 | 0.42** | 136.25 | 21.39 | 0.37** | 237.99 | 36.82 | 0.63** |
| *Poc*-26 | 0.07 | −3.46 | −0.03 | 13.41 | 33.09 | 0.32** | 28.72 | 70.37 | 0.30** |
| *Poc*-5 | 130.40 | 82.63 | 0.83** | 26.36 | 4.34 | 0.25** | 78.54 | 13.03 | 0.87** |
| Average | 482.60 | 22.61 | 0.23** | 1408.22 | 18.79 | 0.24** | 4347.00 | 58.59 | 0.41** |

$^{*}P < 0.05$, $^{**}P < 0.001$.

clonal organisms and are thought to result from the admixture of clonal reproduction and occasional recombination events, though sometimes this is attributed to selection for heterozygotes (Halkett *et al.*, 2005; Stoeckel *et al.*, 2006). Assortative mating can also be invoked in the case of selfing clonal organisms (Stoeckel *et al.*, 2006), such as *P. oceanica*.

Among the 2310 estimates of linkage disequilibrium, the number of estimates with a probability equal or lower to 0.01 (75) did not deviate from the expectation under random distribution (less than 5%). Moreover, the pairs of loci involved in positive linkage disequilibrium were not consistent, nor did the significant values occur preferentially at any locality, supporting the hypothesis of random association of alleles across loci in our data set. In particular, no trend was detected towards higher or more frequent linkage disequilibrium in the samples collected in the central zone. Indeed when analysed on a per population basis, only seven of the 34 populations (six from the western and one from the central zone) exhibited values exceeding 5% (Ro, Eac, Pf, Csm, Ta, Ci, Eg), with a maximum of 13% observed in northern Italy (Ci).

## Genetic structure

The AMOVA revealed that grouping central populations with western ones maximized the proportion of total genetic variance due to differences between groups of populations; this analysis was therefore retained for further interpretation.

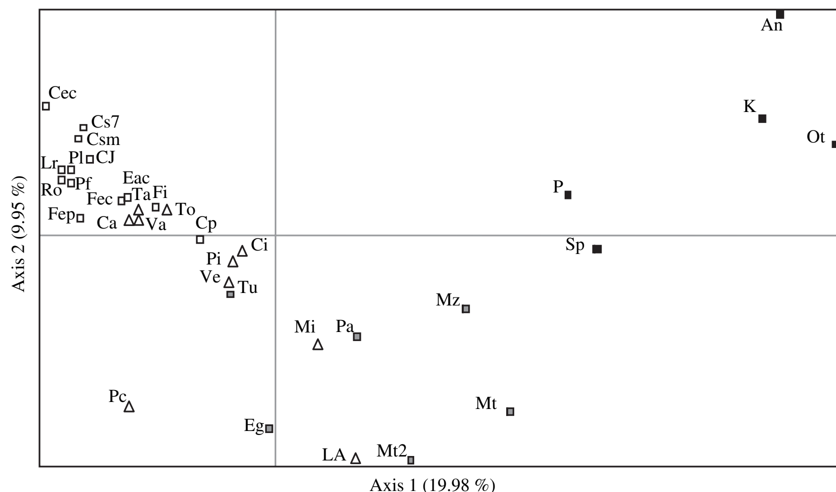


**Figure 2** Correspondence analysis (CA; Benzécri, 1982) performed on the matrix of allelic frequencies of the samples. The inertias of CA-Axis 1 and CA-Axis 2 are 19.98 and 9.95, respectively. Abbreviations for samples are as detailed in Table 1, samples from the western group are represented by white squares (Spain) and triangles (Italy and Corsica), samples from the central and eastern groups are represented by grey and black squares, respectively.


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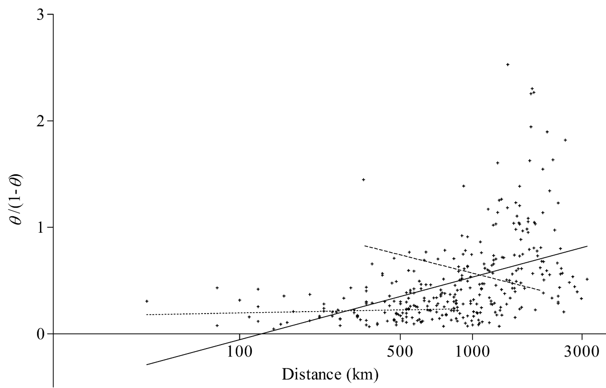


**Figure 3** Estimates of pair wise differentiation [$\hat{\theta}$], transformed ($\hat{\theta}/1-\hat{\theta}$) and plotted against log(geographical distance in kilometres). The regressions are: $y = 0.59x - 1.23$, with $r = 0.47$, Mantel's (1967) normalized statistic $Z = 951.59$ and $P \leq 0.01$ for the whole data set (full line), $y = 0.04x + 0.11$ with $r = 0.01$, $Z = 187.75$ and $P = 0.55$ for the western Mediterranean (broken line), and $y = -0.55x + 2.23$ with $r = -0.18$, $Z = 35.08$ and $P = 0.18$ for the eastern Mediterranean (dotted line).

About 23% of the molecular variance was accounted for by differences between the east and west Mediterranean, 19% by differences among meadows within sub-basins, and 59% by diversity among genets within sampling sites. The partitioning of variance into all three hierarchical levels was highly significant (Table 3), and mostly consistent among loci. However, variance between loci was observed among east–west groups, with lower genetic heterogeneity observed at the loci Po15, Po4-3, Po5-39 and *Poc*-26, and higher heterogeneity at the locus *Poc*-5.

The correspondence analysis illustrates the separation between eastern and western meadows (Fig. 2), the clustering of most samples from the western group (mostly Spain and Italy), and the higher variance among samples from central and eastern groups. A clear west–east gradient appeared along the first axis (accounting for 19.98% of the molecular variance), with the samples from the Siculo-Tunisian Straight (particularly Pantelleria, Marzamemi and the two samples from Malta) exhibiting an intermediate position. It is interesting to note that the sample from northern Sicily (Milazzo), located close to the Messina Strait that separates Sicily from Italy, also shows a somewhat intermediate position along this axis. The second axis (accounting for 9.95% of the variance) mostly reflects the variance within both the central and eastern groups.

All sample pairs showed significant differentiation ($P < 0.05$), with values of $F_{st}$ (data not shown) ranging between 0.04 [between Illetas (Fi) and Es Caló de Oli in the Balearic island of Formentera (Fec)] and 0.71 [between Es Castell in the Balearic island of Cabrera (Cec) and Koper in Slovenia (Ko), where a single genotype was encountered]. At the local scale, no relationship could be observed between genetic and geographical distance, with the absence of a significant relationship between the genetic and geographical distance observed with the Mantel test ($P = 0.23$) at the scale of the Balearic Islands and Spanish continental coasts (less than 200 km; Fig. 3). Some meadows from Formentera Island, separated by 2 to 10 km, were indeed more genetically differentiated ([$\hat{\theta}$] between 0.04, $P < 0.01$ and 0.12, $P < 0.01$) than samples from a meadow from central Spain [El Arenal (Eac)] located 110 km apart (with a [$\hat{\theta}$] value between 0.04, $P < 0.01$ and 0.08, $P < 0.01$). On a larger scale, the Mantel test showed a significant ($P < 0.001$) pattern of isolation by distance across the Mediterranean basin (IBD; Fig. 3), explaining 46.1% of the variance in population differentiation, whereas no significant pattern was detected in the western ($P = 0.55$) and eastern ($P = 0.18$) basins when tested separately (Fig. 3).

When all replicates were included, significant positive autocorrelations, with an average *Sp* statistic ranging from 0.020 to 0.093, were found at the sampling scale in all but two meadows (Campomanes and Punta Fanals; Table 4). When replicate ramets of the same genets were removed, positive autocorrelations were still detected in six of the 15 meadows, with *Sp* statistics of 0.008 to 0.12 (Table 4).

**Table 4** Spatial autocorrelation of genotypic data in 15 *P. oceanica* meadows, including or excluding replicates of the same genotype, based on 12 nuclear loci. Sampling locations, number of samples ($N_s$), number of discriminated genets (MLLs), and *Sp*. Autocorrelation statistics obtained at the ramet (SU level; including all ramets) and genet level (MLL level; including a randomly chosen sample of each genet at each of the 1000 resampling steps), are detailed, with asterisks indicating the significance of these values.

| Sampling localities | $N_s$ | MLLs | *Sp* (SU level) | *Sp* (MLL level) |
|---|---|---|---|---|
| Rodalquilar | 40 | 22 | 0.0267* | 0.0045 ± 0.0064 n.s. |
| Campomanes | 31 | 24 | −0.0062 n.s. | 0.0042 ± 0.0022 n.s. |
| El Arenal-Calpe | 39 | 33 | 0.0205** | 0.0172 ± 0.0021* |
| Las Rotes | 40 | 27 | 0.0562** | 0.0384 ± 0.0010** |
| Punta de Fanals | 38 | 26 | −0.0051 n.s. | 0.0081 ± 0.0022 n.s. |
| Cala Jonquet | 39 | 25 | 0.0928** | 0.0696 ± 0.0038** |
| Port lligat | 40 | 13 | 0.0693** | 0.0067 ± 0.0041 n.s. |
| Es Caló des Oli | 40 | 16 | 0.0499** | 0.0037 ± 0.0047 n.s. |
| Es Pujols | 40 | 30 | 0.0236** | 0.0089 ± 0.0015 n.s. |
| Es Castel | 40 | 7 | 0.0821** | 0.1207 ± 0.0104** |
| Sta María 13 | 35 | 22 | 0.0261** | 0.0054 ± 0.0027 n.s. |
| Sta María 7 | 40 | 24 | 0.0320** | 0.0067 ± 0.0011 n.s. |
| Marzamemi | 38 | 32 | 0.0142** | 0.0077 ± 0.0002* |
| Agios Nicolaos | 40 | 29 | 0.0624** | 0.0471 ± 0.0009** |
| Paphos | 38 | 26 | 0.0285** | 0.0139 ± 0.0030 n.s. |

*$P < 0.05$, **$P < 0.01$; n.s., not significant.

## DISCUSSION

Dinucleotide microsatellites made it possible to detect higher levels of polymorphism in *P. oceanica* than was possible with other markers used in the past (Arnaud-Haond *et al.*, 2005). Using dinucleotide microsatellites, identical ramets could be assigned to the same genet (or multilocus lineage) with high confidence, and a reliable estimation could be made of intra- and interpopulation parameters. Results confirm the general


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pattern of restricted gene flow already suggested by trinucleotide microsatellites (Procaccini *et al.*, 2001, 2002; Ruggiero *et al.*, 2002), and provide more insight into the pattern of high divergence and limited dispersal on various geographical scales. The results presented here reveal genetic structure within individual *P. oceanica* meadows and genetic differentiation among populations on scales ranging from tens of kilometres, up to the great divergence between populations in the eastern and western Mediterranean basins. At the Mediterranean scale, the populations in these two basins have apparently been genetically isolated from one another, which may – if repeated iteratively or maintained over appropriate time-scales – lead to speciation.

### History and evolution of *Posidonia* in the Mediterranean Sea

The lowest levels of genotypic diversity were found in the two samples from the Adriatic Sea and may be attributed, as suggested by Ruggiero *et al.* (2002), to the emergence of this area above sea level during the last glaciations. This implies a recent recolonization by a very small number of individuals followed by predominantly clonal reproduction.

Our study shows that the Siculo-Tunisian Strait is an important genetic boundary between the eastern and western Mediterranean basins, which agrees with findings for a variety of other species (e.g. Quesada *et al.*, 1995; Borsa, 1997; Bahri-Sfar *et al.*, 2000; Nikula & Vainola, 2003). Consideration of the past and present hydrographic regimes in the Mediterranean Sea can help explain such divergence. During the last Pleistocene ice age, exchange between the eastern and western basins was confined to the Siculo-Tunisian Strait, which was much narrower than it is today (Thiede, 1978). After the sea level rose once more, gene flow remained limited to this strait and, to a lesser extent, to the very narrow Siculo-Italian Strait. The water circulation in the Siculo-Tunisian Strait is currently characterized by a constant and unidirectional east-south-east flow of currents arriving from Gibraltar and leaving the coastal zone opposite Tunisia. This contrasts with the rest of the eastern Mediterranean basin, which is characterized by very weak circulation (Pinardi & Masetti, 2000). The pattern of an east–west split between *P. oceanica* populations and the strong genetic structure observed among eastern populations also corresponds well with patterns observed in other species (e.g. Bahri-Sfar *et al.*, 2000).

Other results further suggest that vicariance is a predominant factor shaping the current distribution of genetic diversity of *P. oceanica* at the scale of the Mediterranean Basin, as illustrated by the significantly higher number of alleles observed in the central compared to the western and eastern groups. This observation is valid when comparing the number of alleles per population, as well as the average number of alleles in each of the three groups, and supports the previous interpretation of the east–west cleavage as the result of past isolation (Procaccini *et al.*, 2001, 2002). Secondary contacts between the western and eastern gene pools in the Siculo-Tunisian Strait could explain the significantly higher allelic richness observed in this region. This is supported both by the intermediate position of the central population samples on the first axis of the correspondence analysis, and by the low number of private alleles observed in these populations compared with the number shared with the eastern, western or both of these population groups (Fig. 1).

Given the strong structure and high number of private alleles, it would be interesting to know whether the entities on either side of the divide have initiated or achieved any speciation process during isolation. Phenological (Bussoti *et al.*, 1998) differences between populations in these basins, together with karyotypic discrepancies (Semroud *et al.*, 1992) observed in Algeria, have led researchers to question the taxonomic status of *P. oceanica* across the Mediterranean. No samples from Algeria have been included here (although samples were taken from nearby Tunisia), and the status of the specific Algerian sample, where a distinct karyotype was described, deserves further investigation. However, the genetic divergence between western and eastern Mediterranean populations revealed in the present paper, whilst in agreement with reported phenological differences, is not sufficient to support a revision of the taxonomic status of the species. Speciation, whether achieved or initiated, implies the development of intrinsic factors limiting gene flow (such as pre- or post-zygotic incompatibility) during or immediately after isolation. The presence of such mechanisms limiting gene flow would lead to particular characteristics of the genetic composition in the putative contact zone between the two divergent entities. Among other criteria, the existence of linkage disequilibrium (Barton & Hewitt, 1985) and heterozygote deficiency due to a Wahlund effect can identify such tension zones where differentiation is maintained by total or partial reproductive isolation. However, the repeated heterozygote excess observed in almost all of our sampled populations, including some of the populations located in the putative transition zone, does not support the theoretical expectations. Yet, it is in agreement with other studies on clonal organisms (Ivey & Richards, 2001; Vasseur, 2001; Jump *et al.*, 2003; Prugnolle *et al.*, 2004) and suggests that this index is influenced by other factors, such as heterozygote advantage combined with clonal selection (Schaal & Leverich, 1996; Jump *et al.*, 2003). The absence of heterozygote deficiency in our study cannot therefore be interpreted in terms of mating systems, since the relative influence of opposite forces – such as the Wahlund effect and heterozygosity–fitness relationship – on the inbreeding coefficient cannot be separated in our data. Nevertheless, no significant linkage disequilibrium was encountered in the meadows of the central zone, suggesting the absence of reproductive isolation.

The coexistence of alleles in the Siculo-Tunisian Strait, which are otherwise private to eastern and western populations, implies that there has been sufficient dispersal since the glacial period for the two divergent entities to enter into contact and merge locally. However, the apparent restriction of


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this pattern of secondary contact to a limited region, together with the very low level of diversity in the Adriatic Sea, strongly suggest that the dispersal along the species distribution following the last sea level change has been a slow process and that the system is still not at equilibrium.

The very low speciation rate reported for marine organisms, relative to terrestrial angiosperms (it has been estimated that the number of seagrass species never exceeded 50 since they appeared in the Cretaceous), has been attributed either to the lack of pollinators in the marine environment precluding co-evolutionary processes (an explanation for massive speciation in both insects and terrestrial angiosperms), or to the paucity of barriers to dispersal in the sea (Hemminga & Duarte, 2000; Duarte, 2001). The results derived here suggest that, at least for *P. oceanica*, barriers to dispersal within the biogeographical range of seagrasses may be more important than previously believed.

Finally, in the absence of reproductive isolation, our findings imply that genetic differentiation is maintained by present-day dispersal limitation, thereby underlining a need to understand which factors limit present-day gene flow, and at what scale(s).

## Dispersal at different spatial scales: causes and consequences

At the Mediterranean scale, the IBD observed in the entire sampling set is clearly attributable to the east–west cleavage discussed above, which leads to an apparently spurious correlation between geographical distance and present gene flow, as suggested by Bossart & Prowell (1998; see discussion in Fenster *et al.*, 2003). In our case, no clear relationship between geographical distance and genetic divergence could be demonstrated either at the intrabasin scale or at the local scale. The general trend, even on small geographical scales, was low and apparently stochastically dispersed. As reported for numerous marine taxa from seagrasses (Ruckelshaus, 1998; Reusch, 2002), to invertebrates (Palumbi, 1994; Hellberg, 1996; Duke *et al.*, 1998) and fish (Shulman & Bermingham, 1995; Planes & Fauvelot, 2002), the geographical distance between populations does not appear to be the primary factor influencing the shape of effective dispersal on large scales, and other factors are responsible for the apparently low effective number of migrants ($N_em$) between marine populations.

Dissecting the two parameters shaping $N_em$ estimates may help us understand which factors are involved in limiting gene flow in *P. oceanica*. Firstly, the effective migration rate ($m$) can be limited by physical or biotic factors such as: (1) local wind and currents precluding dispersion or driving seeds towards unsuitable habitats at the time of germination, and (2) low seed germination success due to low turnover of clones and consequent intraspecific competition. Local adaptation does not appear likely to limit the success of dispersal to a great extent since different transplantation experiments involving meadows separated by tens to several hundred kilometres (Balestri *et al.*, 1998; Procaccini & Piazzi, 2001) revealed no varying selection on seed survival. On the other hand, the effective population size ($N_e$) is likely to be small in *P. oceanica* populations, as this is a common feature for aquatic plants and particularly clonal ones (Barrett *et al.*, 1993). Moreover, sparse sexual reproduction (Marbà *et al.*, 2002; Balestri & Cinelli, 2003; Diaz-Almela *et al.*, 2006) or alternative reproductive modalities (Ballesteros *et al.*, 2005), together with the possible variance in reproductive success due to dominance of certain clones (both linked to size differences and to suspected clone-specific flowering) and very high pre-dispersal mortality by abortion and predation (Piazzi *et al.*, 2000; Balestri & Cinelli, 2003), are likely to further reduce $N_e$. Indirect methods for measuring gene flow provide an integrated estimate of many generations of dispersal variance (Slatkin & Arter, 1991). However, in the case of non-equilibrium migration drift, which is likely to apply to *P. oceanica* – particularly under the general pattern of meadow decline occurring in the Mediterranean (Marbà *et al.*, 1996; Moreno *et al.*, 2001; Raniello & Procaccini, 2002; Marbà *et al.*, 2005) – and considering that some clones are aged at more than a millennium (Hemminga & Duarte, 2000), $N_e$ might even be lower than suggested by genetic estimates because of recent decreases. Consequent independent drift in each decreasing population may then explain both the genetic differentiation and the apparent lack of relationship between geographical distance and genetic divergence.

## Autocorrelation and small-scale dispersal

The positive spatial autocorrelation observed (in 13 of 15 meadows) revealed a predominant pattern of spatial aggregation of the ramets of the same genets, as reported for one Italian meadow (Migliaccio *et al.*, 2005). A similar result was reported in stands of the seagrass *Cymodocea nodosa*, using the same statistical analysis on microsatellite data (Alberto *et al.*, 2005; Ruggiero *et al.*, 2005).

The lack of significance in 9 out of 15 meadows after the removal of replicates of the same genet supports the hypothesis of panmixia on the scale at which the sampling was performed in these meadows, although it may be partly due to lower statistical power (40 sample pairs per distance instead of 100 with clonal replicates). In contrast, the existence of strong and positive autocorrelation within the six remaining meadows (located in Spain, Sicily and Greece) suggests that limitation of seed and pollen flow is likely to occur in some meadows on a scale of tens of metres, even though there is apparent potential for larger-scale seed dispersal. The estimated values of the *Sp* statistic at the genet level in these meadows lie between 0.01 and 0.12, and agree with those from a study on a meadow in the Tyrrhenian Sea ($Sp = 0.025$; Migliaccio *et al.*, 2005). Most values range among the highest reported for terrestrial plants but are comparable with those reported for other species exhibiting selfing or mixed mating systems (Vekemans & Hardy, 2004).

The results obtained in the six meadows with significant autocorrelation are comparable to those reported for *C. nodosa*


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(Alberto *et al.*, 2005; Ruggiero *et al.*, 2005), although a priori expectations based on the seed properties of both species differed: *C. nodosa* was expected to exhibit limited dispersal, due to basicarpy and negative buoyancy, whereas fruits of *P. oceanica* float and are thus potentially able to disperse for several weeks under the influence of surface currents and wind (Balestri & Cinelli, 2003). This discrepancy between the *potential* and *realized* dispersal is similar to reports for some pelagic larvae in other marine taxa (Palumbi, 1994). It may be that in *P. oceanica*, the probability of reaching an appropriate environment at the time of germination decreases drastically as a seed disperses away from its meadow of origin (Balestri *et al.*, 1998). Indeed many seeds end up stranded on beaches. Therefore, the seeds incidentally trapped in the rhizome system near their mother ramet may have a higher survival and germination rate than those that disperse further. In addition, the pattern of spatial genetic structure may be accentuated in *P. oceanica* by potential selfing in this monoecious species. Lastly, seed production is remarkably small in most meadows, such that seed gene flow due to dispersal may be limited by low seed production.

In summary, both results at the intermeadow intrabasin scale and at the intrameadow scale show that restriction to dispersal can arise on a scale as small as several metres. It is likely that low effective population size and low dispersal rates produce the general pattern of high genetic divergence observed in this study. Given the general pattern of regression in *P. oceanica* meadows (Marbà *et al.*, 1996; Moreno *et al.*, 2001; Raniello & Procaccini, 2002), the very low apparent dispersal observed at all scales addressed here suggests that we should be concerned about the potential for recolonization of depleted areas.

## CONCLUSION

At the distributional range, historical events and vicariance seem to be the most important drivers of the evolution of spatial genetic differentiation among *P. oceanica* meadows. Although no allopatric speciation has apparently been initiated, the mixing of divergent gene pools from the eastern and western basins still seems to be limited by some physical and/or biological factors and migration–drift equilibrium may still not have been reached. Different factors were revealed by changes in population differentiation observed at different spatial scales. At the intrabasin scale, the vagaries of currents and low turnover could be responsible for the low effective migration rate observed. At the intrameadow scale, very restricted dispersal detected in 40% of the meadows analysed could be explained by the existence of a trade-off between survival and migration.

## ACKNOWLEDGEMENTS

This research was funded by the projects M&MS (EVK3-CT-2000-00044), MedVeg (REN2002-10001-E/MAR), LIFE-Posidonia (2000/NAT/E/7303) of the European Union, and FCT – FEDER from the Portuguese Science Foundation (FCT), and was supported by the network of excellence MARBEF. S.A.H. and F.A. were supported by a post-doctoral and PhD fellowship from the Portuguese Science Foundation (FCT) and the European Social Fund (ESF), E.D.-A. was supported by a PhD grant from the Balearic Government, M.M. was supported by a Ph.D grant from the Seconda Università di Napoli (PhD in Biologia Computazionale). We thank R. Santiago, R. Martínez, E. Álvarez and N. Marbá for assistance during sampling, Fabrizio Campili and all the staff of the Molecular Biology Service of the Stazione Zoologica for technical support during fragment analyses, and F. Bonhomme, G. Pearson and H. McCombie for revising the manuscript.

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## BIOSKETCHES

**S. Arnaud-Haond** is a post-doctoral associate interested in marine evolution and population genetics, **M. Migliaccio** is a PhD student studying the population genetics of seagrasses and **E. Diaz Almela** is a PhD student studying the demography and population genetics of *P. oceanica*.

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Editor: John Lambshead


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