

Disentangling the Influence of Mutation and Migration in Clonal Seagrasses Using the Genetic Diversity Spectrum for Microsatellites

SOPHIE ARNAUD-HAOND, YANN MOALIC, EMILIO HERNÁNDEZ-GARCÍA, VÍCTOR M. EGUILUZ, FILIPE ALBERTO, ÉSTER A. SERRÃO, AND CARLOS M. DUARTE

From the Ifremer, Deep-LEP, Centre de Brest, BP70, 29280 Plouzané, France (Arnaud-Haond and Moalic); the CCMAR, CIMAR-Laboratório Associado, Universidade do Algarve, Gambelas 8005-139, Faro, Portugal (Arnaud-Haond, Alberto and Serrão); the IFSC (CSIC-UIB), Instituto de Física Interdisciplinar y de Sistemas Complejos, Campus Universitat de les Illes Balears, Palma de Mallorca, Spain (Hernández-García and Eguiluz); the Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI (Alberto); the Department of Global Change Research, IMEDEA (CSIC-UIB), Instituto Mediterráneo de Estudios Avanzados, Esporles, Mallorca, Spain (Duarte); and the UWA Oceans Institute and School of Plant Biology, University of Western Australia, Crawley, Western Australia, Australia (Duarte).

Address correspondence to Sophie Arnaud-Haond at the address above, or e-mail: sarnaud@ifremer.fr.

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Abstract

The recurrent lack of isolation by distance reported at regional scale in seagrass species was recently suggested to stem from stochastic events of large-scale dispersal. We explored the usefulness of phylogenetic information contained in microsatellite loci to test this hypothesis by using the Genetic Diversity Spectrum (GDS) on databases containing, respectively, 7 and 9 microsatellites genotypes for 1541 sampling units of *Posidonia oceanica* and 1647 of *Cymodocea nodosa*. The simultaneous increase of microsatellite and geographic distances that emerges reveals a coherent pattern of isolation by distance in contrast to the chaotic pattern previously described using allele frequencies, in particular, for the long-lived *P. oceanica*. These results suggest that the lack of isolation by distance, rather than the resulting from rare events of large-scale dispersal, reflects at least for some species a stronger influence of mutation over migration at the scale of the distribution range. The global distribution of genetic polymorphism may, therefore, result predominantly from ancient events of step-by-step (re)colonization followed by local recruitment and clonal growth, rather than contemporary gene flow. The analysis of GDS appears useful to unravel the evolutionary forces influencing the dynamics and evolution at distinct temporal and spatial scales by accounting for phylogenetic information borne by microsatellites, under an appropriate mutation model. This finding adds nuance to the generalization of the influence of large-scale dispersal on the dynamics of seagrasses.

Subject areas: Population structure and phylogeography; Conservation genetics and biodiversity

Key words: clonality, Genetic Diversity Spectrum, genetic divergence, microsatellites, seagrass, stepwise mutation

The limited dispersal of propagules and gametes across heterogeneous environments is a key component of genetic differentiation in space and time and is considered to be a first step toward speciation, addressed in the early model of isolation by distance (IBD) (Wright 1943). The IBD model continues to provide a framework to analyze and interpret the genetic structure of species across spatial scales (Epperson 1990; Slatkin 1992; Epperson and Li 1996; Rousset 1997;

Planes et al. 2002; Fenster et al. 2003; Vekemans and Hardy 2004). Indeed, understanding the scale dependence of genetic variability within a species range allows inferences on dispersal scales as well as on the influence and strength of the evolutionary processes generating spatial segregation of polymorphism.

Many species providing and shaping habitats on land and in the seas are clonal, including a variety of trees and grasses

and all seagrass and coral species. Recently, this life-history trait has been associated with the observation of low divergence rates or even lack of polymorphism across long regions of nuclear or organellar DNA among congeneric species and lack of polymorphism within species even at the scale of the entire species range (Olsen et al. 2004; Aires et al. 2011). This is thought to result from the long life spans of clonal lineages and overlapping generations potentially resulting in slow evolutionary rates (Aires et al. 2011; Arnaud-Haond et al. 2012). These peculiarities imply that conventional phylogeographic approaches (Coyer et al. 2004; Olsen et al. 2004; Arnaud-Haond et al. 2007a; Alberto et al. 2008; Serra et al. 2010) based on gene diversification have limited power to retrace the large-scale biogeographical events shaping the distribution of polymorphism in these species.

The Genetic Diversity Spectrum (GDS), an individual-based approach summarizing the scale-dependent structure of genetic diversity within a seagrass meadow, allows us to infer the role of several processes (clonality, somatic mutation, selfing, outcrossing, and immigration) in shaping the genetic composition within populations (Rozenfeld et al. 2007; Moalic et al. 2011). In practice, the GDS is represented as the frequency distribution of genetic distances among individuals based on microsatellite markers (Rozenfeld et al. 2007) and adapted from interpopulation distances previously proposed (Goldstein et al. 1995a, 1995b; Slatkin 1995). The GDS illustrates the distribution of the size differences between microsatellite motifs (alleles) similar to the “mismatch distributions” commonly drawn on the basis of gene sequences (Harpending 1994). Thus, provided that the mutation model does not depart significantly from the underlying hypothesis of the Stepwise Mutation Model (SMM), the examination of the GDS across a broad geographic range shows the variable weight of these processes in shaping the genetic structure of individual populations. Analysis of the GDS across seagrass meadows revealed the prevalence of clonality, as well as immigration from distinct meadows, albeit at low frequency (Rozenfeld et al. 2007). Immigration was inferred by the detection of individuals exhibiting a distance to other members of the same deme that largely exceeded the average outcrossing distance, even simulated over a high number of outcrossing generations within the deme (Rozenfeld et al. 2007). Comparison of the within-population GDS of 2 sister brown algal species with contrasting reproductive modes (dioecious vs. selfing hermaphroditic) revealed their clearly different mating systems (Moalic et al. 2011).

Although the GDS has been used to explore the balance of the evolutionary forces that yield the genetic composition of populations and the mating system within seagrass meadows or algal beds, the concept of the GDS could be extended to explore the genetic structure of species across their entire biogeographic range, allowing examination of the scale-dependent weight of the different processes involved.

Here, we extend the individual GDS proposed at the within-population scale by Rozenfeld et al. (2007) to examine processes occurring along the entire biogeographic range. We examine the distribution of genetic distances for 2 clonal

species of seagrass with contrasting mating systems, the long-lived monoecious *Posidonia oceanica* and the dioecious, short-lived, *Cymodocea nodosa*, across their entire biogeographic range. Previous analyses of both species showed high spatial genetic structure with geographical clustering of populations along their distribution range and differentiation of meadows at all spatial scales. Despite this, genetic and geographic distances were not correlated, particularly at regional (i.e., within basins) and local scales (within regions). Lack of IBD at local and regional scales may be explained by 2 contrasting scenarios. First, effective space colonization may lead to random drift by preventing subsequent effective migration even at reduced spatial scales (Becheleer et al. 2010). Second, occasional large-scale dispersal events may explain the general lack of IBD reported in most studies, as suggested by a recent meta-analysis of seagrass population genetics studies (Kendrick et al. 2012). In the first case, it is expected that, provided the analyzed microsatellites predominantly evolve through SMM, the phylogeographic information contained by microsatellites may help retracing past events of colonization or gene flow (Hardy et al. 2003). In such scenario, the GDS based on phylogenetic distance would be expected to show a simultaneous increase of genetic and geographic distance. Contrastingly, if long distance migration events are the main cause, the lack of IBD previously reported on the basis of allelic frequency would persist in a GDS analysis. Also, because the a priori delineation of populations, panmictic units, or biogeographic provinces is not an easy task in clonal organisms (Becheleer et al. 2010, 2014), an interindividual approach—rather than an a priori grouping of individuals in sampling locations—may be a required and informative step toward providing objective spatial or ecological delineation. Here, first, we aimed to describe the evolution of interindividual genetic distances with increasing geographic distances. We examine the accuracy of using phylogenetic information contained in microsatellite size to provide information on scales of differentiation (despite their still-debated mode of evolution and the drawbacks due to possible homoplasy during long-term divergence processes). Second, we aimed to assess the information that a GDS snapshot can yield about the balance among evolutionary forces acting at different spatial scales.

Materials and Methods

Model Species

Posidonia oceanica is a clonal marine angiosperm restricted to the Mediterranean Sea, where it is an essential structural species, developing extensive meadows ranging from 0 to 40 m in depth. It is a very slow-growing organism, with the clones extending horizontally through the growth of rhizomes at approximately 2 cm year⁻¹, developing shoots (ramets, the individual module repeated by clonal propagation) at intervals of approximately 5–10 cm. This monoecious species (i.e., both male and female flowers on the same plant) is characterized by rarely observed episodes of sexual reproduction (Diaz-Almela et al. 2006), important clonality (Arnaud-Haond et al. 2007a),

and extremely long clonal life spans (Arnaud-Haond et al. 2012). Shoots are attached through tough rhizomatic connections, and although some exceptional settlement of drifting shoots has been occasionally observed in very particular patch recruitment conditions, no such event was detected in established meadows despite extensive surveys (Diaz-Almela et al. 2008). These characteristics suggest rare migration among *P. oceanica* meadows, as confirmed by population genetics analysis (Arnaud-Haond et al. 2007a), despite the positive buoyancy of seeds. Individual *P. oceanica* shoots (ramets) live for up to 50 years, and clones have been aged at several thousand years (Arnaud-Haond et al. 2012).

Cymodocea nodosa is also a clonal, but faster growing and short-lived, marine angiosperm distributed both in the Mediterranean and in the Eastern Atlantic, from Senegal to Central Portugal and to the Canaries and Madeira islands. It can be found from very shallow water to depths in excess of 30 m, forming rather dense and large meadows at depth. *Cymodocea nodosa* reproduces regularly in meadows containing a mixture of males and females, but reproduction cannot be completed in monoclonal stands dominated by a single clone (either male or female), as is the case along European Atlantic meadows dominated by single males (Alberto et al. 2008). The seeds are formed at the base of the shoots of females and exhibit negative buoyancy, suggesting rather limited dispersal (Marba and Duarte 1995), and confirmed by genetic spatial autocorrelation (Alberto et al. 2005). However, the occurrence of isolated meadows around oceanic islands shows the ability for occasional large-scale dispersal through the transport of either seeds or, more likely, drifting shoots possibly carrying seeds.

Sampling and Genotyping

The data sets analyzed here were originally developed to study the biogeography of both *P. oceanica* (Arnaud-Haond et al. 2007a) and *C. nodosa* (Alberto et al. 2008) across their biogeographic range. Approximately 40 shoots were sampled in each of 37 localities for *P. oceanica*, ranging from west to east, from the Spanish Mediterranean Coast to Cyprus, encompassing a distance range of 3500–4000 km. A similar sampling was conducted for each of 49 meadows of *C. nodosa*, including the Canaries and Madeira archipelagos and extending along the species range in the Atlantic continental African and European coasts as well as encompassing the entire Mediterranean basin to the Eastern Mediterranean (Cyprus). In all meadows, shoots were collected at randomly drawn coordinates across an area of 20 m × 80 m and 60 m × 14 m for *P. oceanica* and *C. nodosa*, respectively (see Arnaud-Haond et al. 2007a and Alberto et al. 2008 for additional details). Both data sets are archived and available at <http://ifisc.uib-csic.es/EDEN/output.php#data>.

Specimen collection consisted of a portion of the meristem of each shoot that was desiccated and preserved in silica crystals. Genomic DNA was isolated following a standard CTAB extraction procedure (Doyle and Doyle 1987). Two sets of 7 and 9 microsatellite markers were used for *P. oceanica* and *C. nodosa*, respectively (Alberto et al.

2003). Both sets of markers were previously determined to yield the most cost-effective determination of clonal membership (Arnaud-Haond et al. 2005, 2007b). These included loci Po4-3, Po5, Po5-10, Po5-39, Po5-40, Po5-49, and Po15 for *P. oceanica* and Cn2-86, Cn2-38, Cn2-14, Cn2-16, Cn2-18, Cn4-29, Cn2-45, Cn2-24, and Cn4-19 for *C. nodosa*. All loci were perfect dinucleotides, except Cn18-2 that was a compound microsatellite composed of 3 dinucleotide motifs. Finally, each shoot or ramet is characterized by a series of pairs of microsatellite alleles at k loci with $k = 7$ for *P. oceanica* and $k = 9$ for *C. nodosa*. Ramets derived from the same original seed (i.e., belonging to the same genet or clone) will be represented by the same allele combination except for possible somatic mutations occurring during vegetative growth.

Genetic Diversity Spectrum

The GDS was constructed from the frequency distribution of all pairwise interindividual genetic distances for each species, using perl scripts available on request. We used the so-called Rozenfeld's distance (Rozenfeld et al. 2007; Moalic et al. 2011), a distance metric designed to illustrate the effect of clonality and, in contrast with previously available metrics, helps to resolve individuals' origin at an earlier time. This measure of similarity among sampling units yields a distance of zero between identical genotypes (Rozenfeld et al. 2007).

The genotype of a particular sampling unit, called A , is represented as:

$$A = (a_1, A_1)(a_2, A_2) \dots (a_k, A_k),$$

where a_i and A_i are the allele lengths (in number of nucleotides) of locus i in each of the 2 (diploid) chromosomes for k loci. Lower case represents the smaller allele in the pair.

Given a second sampling unit, B , with genotype

$$B = (b_1, B_1)(b_2, B_2) \dots (b_k, B_k),$$

we define a dissimilarity degree between A and B at locus i as:

$$d_i(A, B) = \min(|A_i - B_i| + |a_i - b_i|, |A_i - b_i| + |a_i - B_i|),$$

providing a parsimonious (i.e., minimal) representation of the genetic distance, understood as the difference in allele length, between sampling units A and B . The genetic distance among sampling units is then defined by summing the contributions from each locus (Rozenfeld et al. 2007):

$$D(A, B) = \sum_{i=1}^k d_i,$$

providing the degree of global dissimilarity between A and B (Rozenfeld distance). According to this definition, sampling units from genetically identical individuals (genets or clones) will have a distance of zero.

The distribution of genetic distance D between sampling units is represented as a frequency distribution of all pairwise values, which is referred to as the GDS (Rozenfeld et al. 2007). The GDS is analogous to the frequency distribution

of pairwise differences used on some clonal organism to analyze the effect of clonality and reveal possible somatic mutations or scoring errors (Douhovnikoff and Dodd 2003; Meirmans et al. 2003; Van Der Hulst et al. 2003; Arnaud-Haond et al. 2007b).

A second set of GDS was also built for comparative purpose by using the distance based on shared alleles (SAD, Chakraborty and Jin 1993) based on the proportion of the genome shared, independently of the length differences among microsatellite alleles.

GDS of Random Communities

We tested the shape of the GDS against 2 null hypotheses: 1) panmixia and 2) genetic structure without footprint of colonization and migration history on the spatial distribution of microsatellites (no phylogenetic information borne by microsatellites).

1. Panmixia, implemented via a randomly drawn distribution: Under the hypothesis of global panmixia, the shape and width of the GDS would depend only on the number and level of polymorphism of the markers used and on their random recombination. Panmixia, as a random association of alleles across genotypes by locus, was simulated for both species across their entire range by randomly picking the observed alleles (at observed frequencies) to reconstruct genotypes. In order to take into account the occurrence of clonality in our data set and compare real data with their random equivalent, some of the random panmictic genotypes were chosen within each simulated population to be replicated in the “random data set” the same number of times as the repeated multilocus genotypes (MLGs) were detected in the same population in the “natural data set.”
2. Lack of footprint of migration, implemented via a Structured Allele Shuffling method: As departure from panmixia is observed through several analysis including previous F_{ST} or Bayesian-based reports (Arnaud-Haond et al. 2007a; Alberto et al. 2008), we more precisely aimed to test the departure of GDS from that expected under the hypothesis of a lack of phylogeographic footprint on the spatial distribution of allelic divergence at microsatellite loci. In order to do so, we randomly shuffled allele sizes for each locus and assigned each of the alleles to another one. A brief example: example for a locus with 3 possible alleles of lengths 154, 156, and 160, a random reshuffling may lead to reallocate length 160 instead of 154, 154 instead of 156, and 156 instead of 160. This was generated by a language dictionary that we used to “translate” all genotypes. This Structured Alleles Shuffling maintained the genotypic and genetic differentiation of population based on allelic identity (but not length) and allele frequencies as well as clonality. In this way, the phylogenetic information borne by microsatellite size is randomly distributed among samples, allowing specific tests for the significance of its relation to geographic distance in the original data set.

As a result, in both cases, simulations were made at the ramet level constraining the number of replicated genotypes in

each location to that observed in the real data sets in order to compare real and random or shuffled distributions with the same amount of clonality.

This resulted in the construction of matrices of pairwise distances and GDS for randomly distributed alleles across space. The significance of the deviations between observed and permuted (obtained by simulations under the 2 null hypotheses tested) GDS was tested by Kolmogorov–Smirnov tests in R.

The same test was performed, for comparative purposes, using the shared allele distance and assessing the departure of GDS from that obtained under the null hypothesis of panmixia.

Scale Dependence of the Genetic Diversity Spectra

In order to examine the scale dependence of the GDS, it was dissected into multiple GDSs by sequentially selecting, from the overall matrix used to construct the GDS, all pairwise distances for individuals sampled across increasing ranges of Euclidean geographic distances. In particular, the classes used were 0 km (same locations), 100 km (0 km < distances ≤ 100 km), 500 km (100 km < distances ≤ 500 km), 1000 km (500 km < distances ≤ 1000 km), 2000 km (1000 km < distances ≤ 2000 km), 3000 km (2000 km < distances ≤ 3000 km), 4000 km (3000 km < distances ≤ 4000 km), and >4000 km. These distances were chosen arbitrarily because the number of genetic distance values inside each class is higher than 30 000.

For each species, a Mantel test was then performed for both species and compared assuming dispersal in 1 dimension by plotting genetic versus geographic distance and in 2 dimensions by plotting genetic versus log (geographic distance).

Results

A total of 1541 and 1647 ramets derived from 39 and 60 different meadows were used to build the Spectrum of Genetic Distances of *P. oceanica* and *C. nodosa* across their biogeographic ranges, resulting in 2 373 140 and 2 710 962 pairwise distances that ranged from 0 (for clone mates) to 51 for *P. oceanica* and 31 for *C. nodosa* using Rozenfeld distance. The observed GDSs significantly deviate from the random simulated distribution (Figure 1), showing a significant departure from random association of polymorphism among MLGs across the species distribution ranges for *P. oceanica* distribution (K–S D = 0.40, $P < 0.001$) but not for *C. nodosa* (K–S D = 0.10, $P = 0.99$). Similarly, the departure of shuffled allele distribution (Figure 1) from the real one was significant for *P. oceanica* (K–S D = 0.31, $P < 0.001$) but not for *C. nodosa* (K–S D = 0.26, $P = 0.15$).

The GDS of both species starts at 0 (Figure 1), illustrating the prevalence of clonality. A low frequency of the smaller genetic distance classes was also observed, likely to represent the occurrence of scoring errors, somatic mutations, or both (Arnaud-Haond et al. 2007b; Rozenfeld et al. 2007). Apart from this, the GDSs were fundamentally different for both

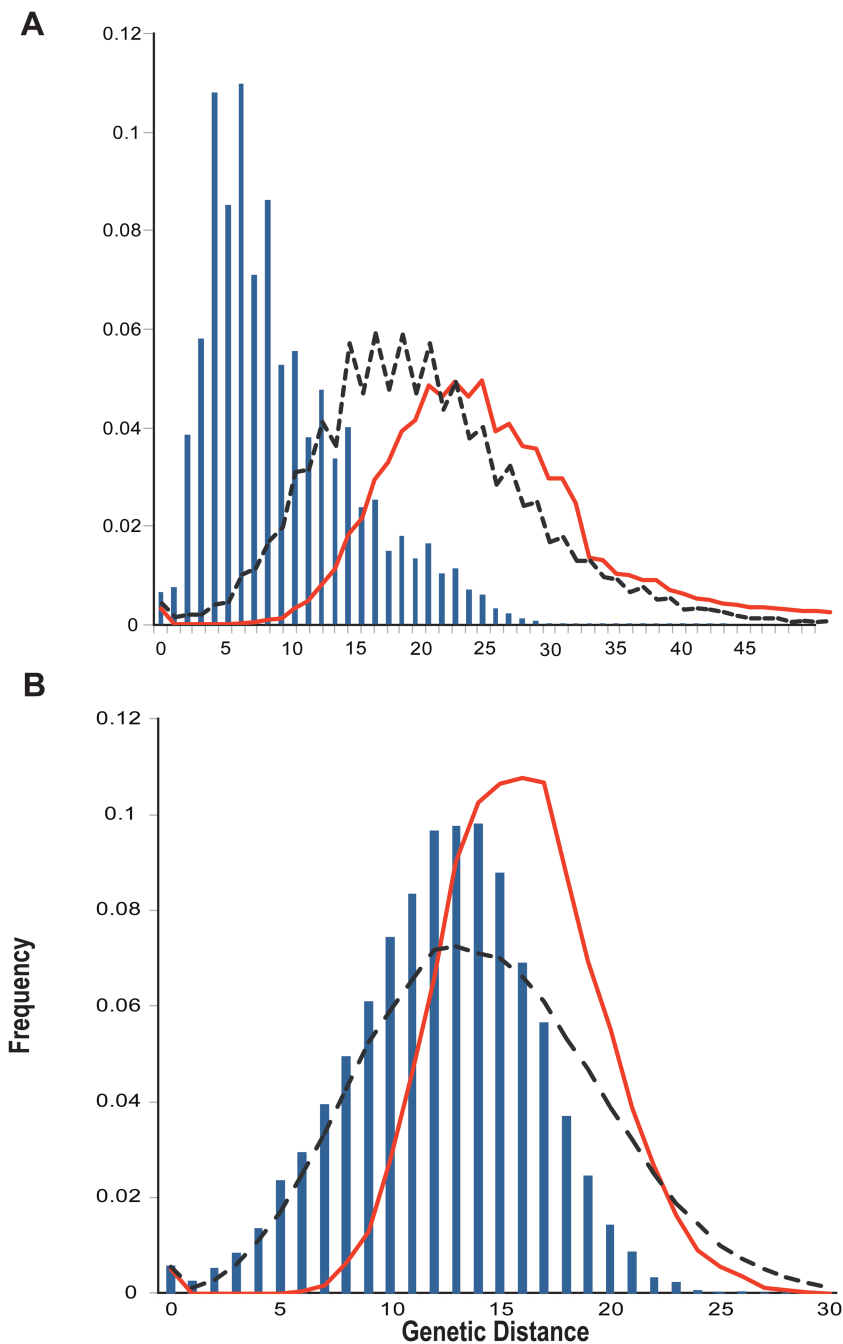


Figure 1. GDS (bar plots) representing the frequency distribution of Rozenfeld interindividual distances at microsatellites (based on microsatellite allele size differences) for *Posidonia oceanica* (A) and *Cymodocea nodosa* (B) across their geographic ranges: from East to West Mediterranean and from East Mediterranean to East Atlantic, respectively. Solid lines represent the randomly drawn distributions, and dashed lines represent the distributions after random shuffling of allele sizes. In both permutation approaches, permuted data set were constrained in order to maintain the same level of clonality (i.e., the same amount of repeated MLG) in each population identical to that in the original data set. Kolmogorov–Smirnov tests were significant for both random permutations and allelic shuffling for *P. oceanica* ($D = 0.40$, $P < 0.001$ and $D = 0.31$, $P < 0.001$, respectively) but for none of the tests for *C. nodosa* ($D = 0.10$, $P = 0.99$ and $D = 0.26$, $P = 0.15$, respectively).

species, with slow-growing and high-longevity *P. oceanica* showing a GDS skewed toward lower genetic distances and composed of several subpeaks, compared with the almost

perfect bell-shape distribution of the spectrum for *C. nodosa* (Figure 1). Contrastingly, the GDSs based on shared allele distance were both strictly unimodal (Supplementary Figure

S1 online). The GDS was significantly departing from the random ones for *C. nodosa* (K–S D = 0.47, $P = 0.046$) but slightly below significance for *P. oceanica* despite similar departure of the curves (K–S D = 0.47, $P = 0.076$), therefore possibly due to limited statistical power.

The scale dependence of the GDS based on Rozenfeld distance was examined by decomposing the GDS into partial GDSs derived from comparisons of pairwise distances of ramets within increasing distance ranges (Figure 2). This exercise further demonstrates important differences between the GDSs of *P. oceanica* and *C. nodosa*. Although most partial GDSs are overlapping, there is a clear linear increase in average genetic distance with increasing geographic distance in

P. oceanica (Figure 2A). The larger pairwise genetic distance classes were dominated by pairs of ramets located at distant locations across the Mediterranean basin. The same tendency, but with a much narrower shift in genetic versus spatial distance, was obtained for *C. nodosa*, where partial GDSs show a greater overlap (Figure 2B). Mantel tests based on bins of those sequential GDS were highly significant (Table 1) for both *P. oceanica* ($R = 0.83$ and 0.79 , $P < 0.001$, in 1D and 2D spaces, respectively) and *C. nodosa* ($R = 0.61$ and 0.48 , $P < 0.01$, in 1D and 2D spaces, respectively). Both analyses confirm the scale dependence of the genetic structure for both species in a pattern closer to a 1D dispersal model and its greater strength in *P. oceanica*.

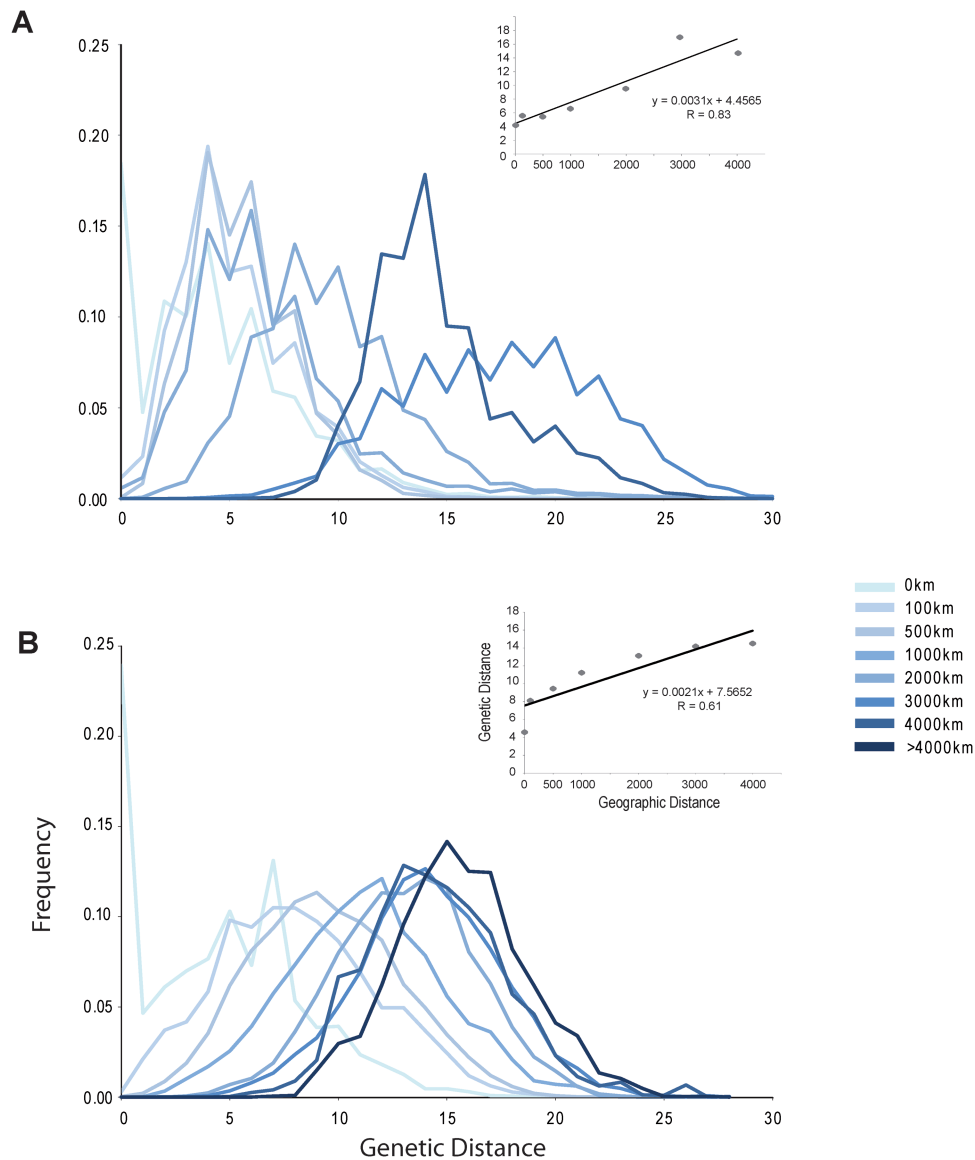


Figure 2. Decomposition of the GDS across distinct sets of geographic distances, illustrating the change in mean Rozenfeld genetic distances (based on microsatellite allele size differences) with geographic distances for *Posidonia oceanica* (A) and for *Cymodocea nodosa* (B). The insets show the regression corresponding to the significant Mantel tests performed on bins of geographic distances (Table 1).

Table 1 Results of the Mantel tests for both species with Rozenfeld and allele shared distances plotted against geographic distances or their log values to fit a 1D or 2D model

	<i>Posidonia oceanica</i>		<i>Cymodocea nodosa</i>	
	1D	2D	1D	2D
Rozenfeld distance	0.83**	0.79**	0.61*	0.48*
Allele shared distance	0.75**	0.62***	0.46*	0.32

Significance of Mantel tests is indicated with * $P < 10^{-2}$, ** $P < 10^{-3}$, and *** $P < 10^{-4}$.

The dissection of GDS based on SAD also showed an evolution of genetic distance with geographic ones (Supplementary Figure S2 online). However, this was true only up to a certain point as the peaks at large geographic distance are largely overlapping. Mantel tests showed significant but less strong relationships (Table 1), with the exception that for *C. nodosa*, no significant relationship was detected in a 2D scheme.

Discussion

Our GDS analysis opens new perspectives concerning the main evolutionary forces affecting the ecology and evolution of seagrasses, emphasizing their mating system. The recurrent lack of overall IBD at the regional scale reported for several seagrass species, including *P. oceanica* (Arnaud-Haond et al. 2007a) and *C. nodosa* (Alberto et al. 2008), was recently proposed to stem from occasional events of stochastic large-scale dispersal (Kendrick et al. 2012). This led the authors to suggest the importance of large-scale dispersal for the ecology and evolution of those species. Results presented here offer an alternative or complementary hypothesis by revealing the existence of an IBD pattern observed when taking into account the phylogenetic information borne by microsatellites. The results indicate the dominant role of mutation–drift versus a negligible influence of contemporary effective migration. Such a pattern may result from the strong influence of historical colonization patterns, followed by local recruitment and accumulation of divergent mutations through drift, and implies low dispersal and strong influence of clonality on the dynamics and evolution of some seagrass species. Differences in the patterns observed between both species also suggest the influence of life-history traits and/or history on the weight of these distinct scenarios.

The GDS of the 2 seagrass species examined here indeed departed significantly from randomness with Rozenfeld distance only for *P. oceanica*, and with SAD only for *C. nodosa*, revealing distinct but significant patterns of genetic structure across their distributional ranges. This departure was particularly pronounced for *P. oceanica*, which exhibited successive peaks in the Rozenfeld-based GDS (Figure 1A), revealing a system of populations substructured into divergent clusters of individuals, despite the lack of IBD previously reported on the basis of F_{ST} (Arnaud-Haond et al. 2007a). In contrast, the GDS for *C. nodosa* showed a profile typical of a normal distribution (except for the clonality appearing at 0) with a

single mode at high genetic distances. This reveals a lack of hierarchical divergence (Figure 1B), even though differentiation occurs, as revealed by the departure from randomness observed for the SAD-based GDS and Mantel tests using partial GDS either based on Rozenfeld or SAD distances. Indeed, for both species, decomposing the GDSs into partial GDSs at increasing spatial scales (Figure 2) revealed regular and positive relationships between genetic and geographic distances, reflecting dispersal limitation at small spatial scales, and the history of divergence of clones across their distribution ranges at larger scales. This corresponds to an IBD model applicable even at the intrabasin scale (Figure 2, Supplementary Figure S2 online, Table 1). This pattern contrasts with the lack of evidence for intrabasin IBD (within marine regions bordered by the Siculo-Tunisian or the Gibraltar straits) reported for the same populations on the basis of F_{ST} differentiation levels, for *P. oceanica* (Arnaud-Haond et al. 2007a) and *C. nodosa* (Alberto et al. 2008), where the only indices of a relationship between geographic and genetic distance emerging at global scale was due to biogeographic breaks.

The increasing genetic divergence with spatial distance revealed by the partial GDSs provides evidence of IBD and reveals very low effective dispersal in these species. The lack of correlation between high genetic differentiation and geographic distances at regional scale observed in previous F_{ST} -based studies was proposed to be the result of the strong influence of genetic drift and stochastic migration of sexually produced propagules, with little influence of geographic distance on the success of dispersal events (as suggested in Kendrick et al. 2012). However, F_{ST} estimates based on the frequency of alleles in a strongly structured system, as in the present case, may soon saturate the maximum level of divergence that can be revealed, resulting in an asymptotic value of the curve describing the increase of F_{ST} with the divergence time (Hutchison and Templeton 1999). In such a saturated profile, only very slight differences, possibly lower than the standard error of the estimates, are expected among the mean F_{ST} located beyond the asymptote threshold. This is accompanied by a variability in the point of equilibrium mutation–migration–drift depending on the markers and models used. Such saturation of frequency-based distances using hypervariable markers impedes the accurate discrimination of distinct levels of differentiation beyond a certain threshold, which may have been overcome in the case of highly differentiated meadows. This threshold is highly dependent on the variability of the markers used (Beaumont and Pether 1996; Hedrick 1999; Jost 2007, 2008), with more variable markers usually leading to earlier saturation. This phenomenon may be responsible for the apparent inconsistency between GDS and F_{ST} analyses, with regard to the relationship between genetic and geographic distances at the regional scale. Indeed, it becomes a challenge to distinguish high levels of differentiation from even higher ones on the basis of F_{ST} estimates prone to a nonnegligible variance beyond a certain threshold, contrary to the observed increase reported here for partial GDSs (Figure 2). Some corrections have been proposed using distance metrics based on allele frequency to obtain estimates that would not depend on

the level of variability within demes, such as the recently proposed D or G_{st} (Hedrick 1999, 2005; Jost 2007, 2008). The main condition for the reliable performance of these modified metrics seems to be a relatively higher influence of mutation versus migration on the mutation–migration–drift equilibrium specific to study the systems in question. Results obtained here (Figure 2) suggest that these conditions are fulfilled in the 2 seagrass species studied but additionally support the existence of relevant phylogeographic information in microsatellites at least for the long-lived *P. oceanica*.

The geographic coherence of the divergence observed here from the information contained in microsatellites across species distributional ranges indeed confirms that, under a relatively higher influence of mutation versus migration on the mutation–migration–drift equilibrium, an additional benefit may be obtained by using not only the allelic frequencies but also the potential phylogenetic information contained in the difference in number of motifs borne by distinct alleles (Goldstein et al. 1995a, 1995b; Slatkin 1995; Takezaki and Nei 1996; Goldstein and Pollock 1997). Estimates based on allelic frequencies alone assume an Infinite Allele Model, with mutation giving rise to a new allelic state uncorrelated with the mutated allele, although there is evidence that this model is not accurate for most microsatellite loci (Schlotterer 2000). The use of differences in microsatellite length can, therefore, reveal phylogeographic patterns given an underlying mutation model (SMM) possibly closer to that actually operating on these markers (Rousset 1996; Goodman 1997; Ballou and Goudet 2002; Hardy et al. 2003). Compared with the estimators D or G_{st} , the GDS approach used here accounts for the high level of diversity, while simultaneously taking advantage of meaningful information conveyed by microsatellite alleles that depends on the relative influence of mutation in the mutation–migration–drift equilibrium, and also on the accuracy of the mutation model assumed, here the SMM.

The analysis of partial GDSs presented here not only confirms the very strong structure revealed by F_{ST} for both species but also reveals coherence between spatial and genetic distances that remained hidden in previous analyses that ignored the phylogenetic information contained in microsatellite length variation. The species studied here have contrasting reproductive modes and dispersal biology; fruits of *P. oceanica* remain buoyant over several weeks and have a rather high dispersal potential (Balestri and Lardicci 2008), whereas seeds of *C. nodosa* are negatively buoyant and remain attached to the mother plants until germination. On the other hand, ramets of *C. nodosa* are more likely to fragment and provide a midrange dispersal mean than the much thicker rhizomes of *P. oceanica*. Yet despite their biological differences, reflected in different GDS patterns, both species show a comparable high spatial genetic structure, demonstrating that the dispersal potential is not fully realized for either of them. This limitation seems particularly important in *P. oceanica* where not only the Mantel tests but also the GDS itself shows departure from random expectations, suggesting an even stronger influence of mutation and a greater limitation to gene flow in this long-lived species than in *C. nodosa*. The correlation between genetic divergence and geographic distance reported

here may result from the high influence of mutation, rather than contemporary migration, on the pattern of spatial distribution of polymorphism among populations. This holds even at the regional scale as this shift in the distribution of genetic distances is already evident at scales of several hundreds of kilometers (Figure 2). Extant meadows may, therefore, be the result of ancient stepwise colonization events, followed by a rather allopatric evolution with very few migration events and a dominant clonal space occupation. The initial pattern induced by the colonization process is thus preserved and mostly determined by distance to the source. Such a scenario is supported by the sparse flowering (Diaz-Almela et al. 2006, 2009) and very low sexual output observed for *P. oceanica* (Balestri et al. 1998; Balestri and Cinelli 2003; Diaz-Almela et al. 2006, 2009) across the Mediterranean, in line with the limited (Arnaud-Haond et al. 2005, 2007a) though still likely overestimated (Arnaud-Haond et al. 2007b) clonal diversity in *P. oceanica* meadows. It is also in line with extremely low expected dispersal linked to the negative buoyancy of seeds in *C. nodosa* and strikingly contrasted patterns of genotypic diversity in neighboring meadows for both species (Alberto et al. 2006, 2008; Arnaud-Haond et al. 2007a). This initial colonization strategy followed by predominantly local and clonal recruitment was proposed by Eriksson (1993) to be one of the extreme possible life-history strategies for clonal plants, referred to as Initial Seedling Recruitment (ISR) as opposed to Repeated Seedling Recruitment involving the occurrence of periodical events of seed recruitment. Such an ISR pattern was diagnosed for several seagrass species on the basis of spatial autocorrelation analysis (Alberto et al. 2005; Bechele et al. 2010) and is equivalent to the persistent imprint of initial founder effects described in nonclonal populations followed by self-recruitment over millennia (e.g., Neiva et al. 2012). The ISR seems to imprint genetic composition over large time scales as the divergence observed in line with geographic distances suggest that the population system would be “crystallized” since the last (re)colonization of present day meadows, resulting in a genetic structure mostly driven by clonal growth, competition, and drift after a “stepping-stone” colonization process. This would also be in agreement with recent findings of extreme life span for some *P. oceanica* clones (Arnaud-Haond et al. 2012), the domination of vast areas by single clones of *C. nodosa* (Alberto et al. 2001, 2008), and reports of extreme generation time for clonal organisms (Oinonen 1969; Vasek 1980; Laberge et al. 2000; van der Merwe, Spain and Rossetto 2010). There is a peak inversion between the 2 last distance bins in *P. oceanica* (Figure 2 and to a lower extent in Supplementary Figure S2 online), which seems to contradict the general interpretations given before and based on geographic distance alone. This change in ordering is mostly due to an inversion of distance among meadows sampled in Greece and Cyprus compared with those present in central or western part of the Mediterranean. This may either be attributed to historical pathways of migration or (re) colonization among Mediterranean basins or to differential effects of mutation and drift in the populations in those 2 regions.

Finally, such an ISR strategy has been shown to result in genetic differentiation at all geographic scales the seagrass

meadows studied, including among neighboring quadrates within meadows, rendering the genetic concept of population cumbersome (Becheler et al. 2010). This challenges the a priori delineation of sets of samples to be pooled or not, to perform summary statistics, whereas the use of the GDS to identify scales of differentiation does not require such a priori delineation and can help in recognizing the breaks corresponding to the distance below which genetic structure is mostly due to ISR and consequent genetic patchiness and above which divergence occurs as a result of mutation accumulation through longer term isolation. This threshold appears to occur beyond a few hundred kilometers for both species (Figure 2).

This work shows that, when microsatellites exhibit a mutation model close enough to stepwise, the GDS analysis is a useful complementary tool to analyze the scale dependence of evolutionary processes and unravel the biogeography of some species across a large part of their biogeographic range. This is particularly useful for clonal species for which many other approaches fail due to the exceedingly low evolution of genes classically used for phylogeographic reconstruction. While showing that useful phylogenetic information may also be borne by microsatellites, as previously emphasized (Goldstein et al. 1995a, 1995b; Slatkin 1995; Rousset 1996), the evidence presented here also support the prerequisite of a predominant importance of mutation to use allele length at microsatellites in a phylogenetic framework.

Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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