Molecular Ecology (2005) **14**, 2669–2681 doi: 10.1111/j.1365-294X.2005.02640.x

Within-population spatial genetic structure, neighbourhood size and clonal subrange in the seagrass *Cymodocea nodosa*

FILIPE ALBERTO,* LICÍNIA GOUVEIA,* SOPHIE ARNAUD-HAOND,* JOSÉ L. PÉREZ-LLORÉNS,† CARLOS M. DUARTE‡ and ESTER A. SERRÃO*

**CCMAR, CIMAR-Laboratório Associado, University of Algarve, Campus de Gambelas, 8005–139 Faro, Portugal, †Area de Ecología, Universidad de Cadiz, Facultad de Ciencias del Mar y Ambientales, 11510 Puerto Real, Cadiz, Spain, ‡IMEDEA (CSIC-UIB) Instituto Mediterraneo de Estudios Avanzados, C/Miquel Marques 21, 07190 Esporles, Mallorca, Spain*

Abstract

The extent of clonality within populations strongly influences their spatial genetic structure (SGS), yet this is hardly ever thoroughly analysed. We employed spatial autocorrelation analysis to study effects of sexual and clonal reproduction on dispersal of the dioecious seagrass *Cymodocea nodosa*. Analyses were performed both at genet level (i.e. excluding clonal repeats) and at ramet level. Clonal structure was characterized by the clonal subrange, a spatial measure of the linear limits where clonality still affects SGS. We show that the clonal subrange is equivalent to the distance where the probability of clonal identity approaches zero. This combined approach was applied to two meadows with different levels of disturbance, Cadiz (stable) and Alfacs (disturbed). Genotypic richness, the proportion of the sample representing distinct genotypes, was moderate (0.38 Cadiz, 0.46 Alfacs) mostly due to dominance of a few clones. Expected heterozygosities were comparable to those found in other clonal plants. SGS analyses at the genet level revealed extremely restricted gene dispersal in Cadiz (Sp = 0.052, a statistic reflecting the decrease of pairwise kinship with distance), the strongest SGS found for seagrass species, comparable only to values for selfing herbaceous land plants. At Cadiz the clonal subrange extended across shorter distances (20–25 m) than in Alfacs (30–35 m). Comparisons of sexual and vegetative components of gene dispersal suggest that, as a dispersal vector within meadows, clonal spread is at least as important as sexual reproduction. The restricted dispersal and SGS pattern in both meadows indicates that the species follows a repeated seedling recruitment strategy.

Keywords: clonal plant, clonal subrange, *Cymodocea nodosa*, microsatellites, seagrass, SGS

Received 1 February 2005; revision received 15 April 2005; accepted 6 May 2005

Introduction

Genetic structure can be defined as the development of a nonrandom distribution of alleles at a given spatial scale resulting from limited dispersal, selection, genetic drift and population history. An important concept related to dispersal is isolation by distance, which predicts the expected pattern of spatial genetic structure (SGS) under restricted dispersal and local genetic drift (Vekemans & Hardy 2004). The amount and scale of gene flow determines the role of local adaptation and population SGS in the evolutionary process (Wright 1977; Fenster *et al*. 2003). In plants, SGS is the result of the joint effect of pollen and seed dispersal and, for the numerous plant species exhibiting clonality, of the pattern of clonal growth. Indeed, clonality is expected to greatly influence the patterns of SGS, both because of the expected aggregated distribution of clone mates with an identical genotype, and because clonal growth is also a component of spatial dispersal (Gliddon *et al*. 1987). The strength of the influence of clonality on SGS will depend on the type and rate of clonal growth (Marbà & Duarte 1998), intermingling among clones, fragmentation and the lifespan of the genets. For example if the clone becomes fragmented and is present at long distances from the initial seedling germination site, then clonal reproduction increases the

Correspondence: Ester A. Serrão, Fax: +351 289818353; E-mail: eserrao@ualg.pt

genet dispersal (Chung & Epperson 1999, 2000; Chung *et al*. 2000). Species or stages that exhibit a 'guerrilla' type of clonal growth (irregular shape and widespread ramets: Lovett Doust 1981) are expected to show high clonal intermingling and dispersal. Conversely, a 'phalanx' strategy (regularly shaped radiating circles of densely clumped ramets) will lead to clone mate clustering and consequently to increased SGS. A key question specific to clonal organisms is the effect of clonality on SGS, i.e. the identification of the spatial scales over which clonal processes affect the genetic structure of the population, which we hereafter refer to as the clonal subrange of the SGS. Despite this, some studies purposely increase the porosity of the sampling program so as to avoid any effects of clonality on the resulting depiction of the SGS (i.e. selecting a minimum sampling distance greater than the expected spread of the clones). This approach results in loss of information on both the role of clonality as a driver of SGS, and on the SGS at small spatial scales.

Spatial genetic autocorrelation analysis examines the genetic relatedness between pairs of individuals with regard to their relative positions in space (cf. Epperson 2003 and Vekemans & Hardy 2004). Theoretical models of isolation by distance predict patterns of SGS at drift–dispersal equilibrium, and recent theoretical and methodological advances allow new inferences about gene dispersal and neighbourhood size (Rousset 1997; Hardy & Vekemans 1999; Vekemans & Hardy 2004). For neutral genetic markers the expected outcome of SGS on spatial autocorrelograms is a linear decrease in the mean genetic kinship coefficient with the logarithm of spatial distance for two-dimensional populations. The slope of the regression equation describing this decline can be used to estimate SGS parameters such as gene dispersal and Wright's 'neighbourhood size' (Fenster *et al*. 2003).

Effective sexual recruitment is the result of pollen and seed dispersal, seed germination and establishment, and seedling growth. Recruitment behaviour and genet dynamics in clonal plants have been described in an idealized and simplified way by Eriksson (1993, 1997) as a continuum from 'repeated seedling recruitment' (RSR) into adult populations, and unique 'initial seedling recruitment' (ISR) at the beginning of population history. In the RSR strategy seedlings recruit regularly within stands of established adults, whereas in the ISR strategy they only establish during the initial colonization period, and further development of the meadow is due to clonal growth (Eriksson 1993, 1997). It has been hypothesized that repeated seedling recruitment would be more common in clonal marine plants than in their terrestrial counterparts (Inglis 2000), since the micro-environment provided by established meadows may increase seedling survival (Terrados 1993).

Here we analyse the SGS and clonal structure of a dioecious seagrass (*Cymodocea nodosa*) in two meadows by means of spatial autocorrelation using pairwise kinship coefficients estimated from microsatellite alleles and four different methods of analysing the data. Based on *C. nodosa* seed morphology and position, we expected sexual dispersal to be weak and the meadows to exhibit 'repeated seedling recruitment', while the high vegetative growth rate of this species suggests that clonal propagation should be an important dispersal vector. Therefore, our objective in the present study was to analyse the relative importance of clonal and sexual dispersal. To achieve this, we evaluated the potential of spatial autocorrelograms to estimate dispersal parameters such as (i) the neighbourhood size and gene dispersal, and (ii) the clonal subrange; a measure of the spatial scale at which the probability of clonal identity is near zero (Harada & Iwasa 1996; Harada *et al*. 1997), estimating the spatial range over which clonality directly affects SGS.

Materials and methods

Model species

Cymodocea nodosa (Cymodoceaceae) is a dioecious, rhizomatous seagrass (Hemminga & Duarte 2000) that exhibits fast clonal growth, with maximum linear clonal extension rates in excess of 2 m/year (Duarte & Sand-Jensen 1990). It occurs throughout the Mediterranean basin and in the North Atlantic from central Portugal to Cap d'Arguin in Senegal, as well as in the Canary Archipelago and the Madeira Islands. *Cymodocea nodosa* exhibits basicarpy, producing two seeds at the base of the female shoots where, in the absence of disturbance, they remain buried under the sediment until germination occurs (Buia & Mazzella 1991). This suggests highly restricted seed dispersal, and although there are anecdotal observations of seeds cast upon the shore and seeds transported by positively buoyant detached shoots, these mechanisms appear to represent rare dispersal events. To date, no direct estimates of seed dispersal are available for this species and the potential for pollen dispersal remains undetermined. While *C. nodosa* seedlings have been found at the periphery of established patches, the probability that they could grow and develop a new patch is estimated at about 10% (Duarte & Sand-Jensen 1990), although this figure may be higher inside established meadows (Terrados 1993).

Study sites and sampling

Two contrasting meadows, separated by over 1000 km along the Spanish coast, were sampled in June 2003 to examine the spatial structure of *C. nodosa* microsatellite genotypes (see Fig. 1). The Cadiz population occurs on the southwest margin of the tidal bay of Cadiz in the Atlantic, where it extends from 0.5 to 3 m water depth. The Alfacs population grows in the tideless Alfacs Bay in the

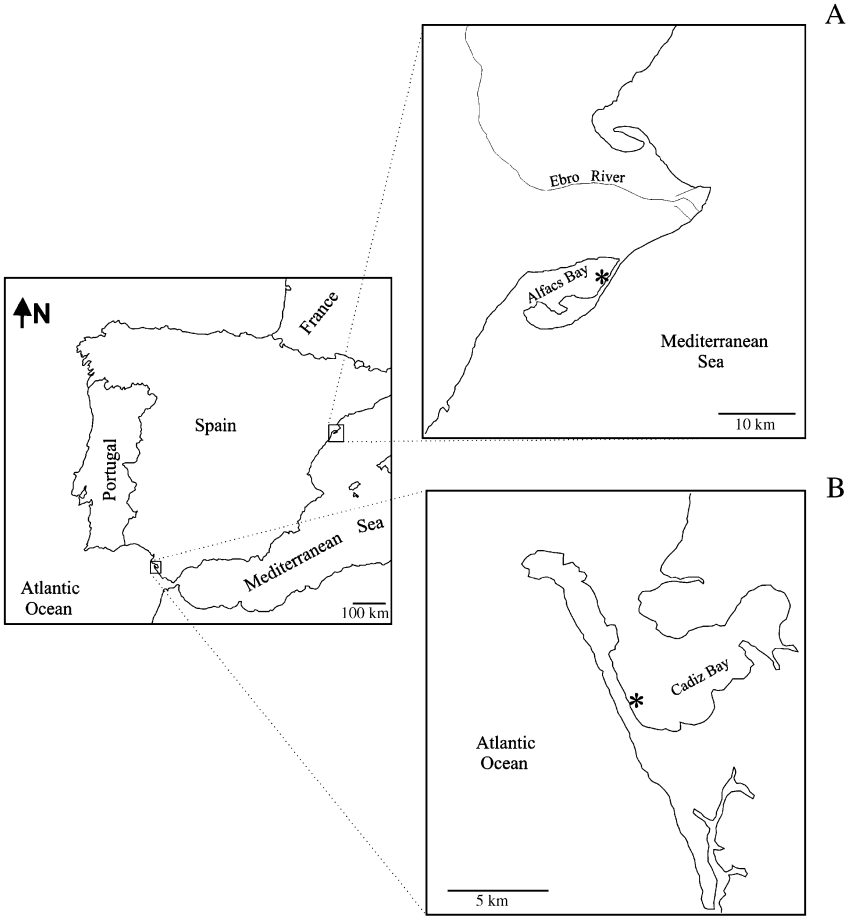

Fig. 1 Sampling sites of *Cymodocea nodosa* for spatial genetic structure analyses. (A) Alfacs Bay in the Mediterranean coast of Spain, associated with the Ebro River delta. (B) Cadiz Bay in the South Atlantic coast of Spain.

Mediterranean, at 0.5 m depth. The two populations are also subject to contrasting disturbance regimes: Alfacs Bay is periodically disturbed by the migration of subaqueous dunes (Marbà *et al*. 1994; Marbà & Duarte 1995) and the local landscape, dominated by *C. nodosa* patches, is characterized by an extinction–recolonization balance (Vidondo *et al*. 1997). In contrast, in Cadiz Bay the *C. nodosa* meadows are continuous and apparently undisturbed.

At each site, sampling was performed along a grid of 20×38 m. The internal grid spacing was 2 m yielding a total of 220 sampling units per population. For each sampling unit, the meristematic portion of 3–5 shoots, belonging to the same rhizome/genet, was preserved and dried on silica crystals before transportation to the laboratory.

Microsatellite genotyping

After DNA extraction (Doyle & Doyle 1988) samples were genotyped for nine microsatellite loci (Alberto *et al*. 2003). Three polymerase chain reaction (PCR) multiplexes with fluorescently labelled primers (MA, MB and MC) followed by two electrophoresis multiplexes (MA + MC and MB) were sufficient to analyse all loci on an ABI 377 automated sequencer using the GENESCAN software (Applied Biosystems). Approximately 10 ng of DNA were amplified in a 15-μL volume, containing 60 μM of each dCTP, dGTP, dATP and dTTP, 2 mM of $MgCl_2$, 200 mM Tris-HCl (pH 8.4), 500 mM KCl and 1 U *Taq* DNA polymerase (Invitrogen, Life Technologies). Each reaction contained one of the following multiplexes of fluorescently labelled *C. nodosa* microsatellite primers: (MA) Cn2–86/6-FAM, Cn2–38/HEX and Cn2–14/6-FAM; (MB) Cn2–16/HEX, Cn2–18/6-FAM, Cn4–29/NED and Cn2–45/6-FAM; (MC) Cn2–24/NED and Cn4–19/NED. Individual primer concentration ranged from 0.06 to 0.23 μM. Cycling conditions consisted of an initial denaturing step of 4 min at 94 °C, followed by 24 cycles of 'touchdown' PCR consisting of 30 s at 94 °C, 30 s at 55 °C (reduced by 0.2 °C in each subsequent cycle), and 30 s at 72 °C, 10 additional cycles consisting of 30 s at 94 °C, 30 s at 50 °C and 40 s at 72 °C, and a final elongation step at 72 °C for 10 min.

Clone identification

Observed identical multilocus genotypes (MLGs) can either be the result of sampling the same clone/genet at

two different spatial coordinates, or two different genotypes originated by two distinct sexual reproduction events but sharing the same alleles for all genotyped loci (Arnaud-Haond *et al*. 2005). The probability of encountering the latter depends on the population frequencies for the alleles in that genotype and the number of loci used to fingerprint samples. To address this issue, we estimated the probability of a given multilocus genotype occurring n times as a consequence of different sexual reproduction events (P_{sex}), according to Parks & Werth (1993). Detailed description of P_{sex} estimation and genet assignment using an appropriate set of markers is reported elsewhere (Arnaud-Haond *et al*. 2005). Once clones were identified genotypic richness was estimated for each site according to Dorken & Eckert (2001) as:

$$R = \frac{G - 1}{N - 1} \quad \text{(eqn 1)}$$

Where G is the number of distinct genotypes and N the sample size. The distribution of clone size was described using the distance between the farthest clone mates as a conservative estimate of the linear size of the clone. In clonal plants this distribution is typically skewed with only a few clones having large dimensions. Hämmerli & Reusch (2003a) found for the seagrass *Zostera marina* that clonal size increased with heterozygosity, suggesting that more outbred genets would be better competitors for space occupation. We examined the hypothesis of a relationship between genet heterozygosity and clone size (number of clonal replicates) using a Monte Carlo simulation provided by the program CLONALITY version 1 (Prugnolle *et al*. 2004). This program tests if MLGs repeated in the sample (i.e. clonal growth) have an increasing effect on population heterozygosity, resulting in a decrease of the inbreeding coefficient F_{IS}. The program first estimates F_{IS}, using Weir & Cockerham's (1984) unbiased estimator f, without removing repeated MLGs (sample N) and detects how many MLGs have multiple copies and how many copies for each of these. It proceeds by reducing the data to a single copy for each MLG (sample U), and a new sample is then generated (sample R1) by amplifying randomly chosen genotypes of sample U so that the sample size and the amount of repetitions of multilocus genotypes are kept identical to those found in sample N (see Fig. 1, Prugnolle *et al*. 2004). The procedure is repeated 5000 times (samples R1 to R5000) and a corresponding f_{Ri} is computed each time (f_{R1} to f_{R5000}). A P value is obtained by computing the proportion of times that $f_{Ri} \leq f$.

Population genetic statistics

Allele frequencies, expected heterozygosities (H_E) and inbreeding coefficients (f) were estimated using the software GENEPOP (Raymond & Rousset 1995). Hardy–Weinberg equilibrium and genotypic linkage disequilibrium (using a single copy per genet) were tested for each population using the exact Hardy–Weinberg test (Weir 1990) and the Fisher exact test, respectively, both available in GENEPOP.

Spatial genetic structure

Two main types of analyses were performed with both data sets, one with the repetitions of MLGs kept throughout, and another using a single copy per MLG; they are hereafter called ramet- and genet-level analyses, respectively. At the ramet level one tries to characterize the potential amplifying effects of clonality on SGS, created by the genetic correlation between clone mate pairs. The genet-level analysis circumvents the latter problem; however, clonality is a component of dispersal and so affects the observed SGS pattern even when repeated MLGs are removed.

The genetic co-ancestry between pairs of individuals can be summarized over a range of distance intervals in terms of multilocus estimates of kinship (F_{ij}). In order to do so, we used a kinship coefficient used in Loiselle *et al*. (1995) and implemented in the software SPAGEDI (Hardy & Vekemans 2002). Average kinship coefficients were estimated for the following distance classes: 0–2; 2–4; 4–6; 6–8; 8–10; 10–12; 12–14; 14–16; 16–18; 18–20; 20–25; 25–30 and 30–45 m. Correlograms were constructed by plotting mean pairwise kinship coefficients as a function of spatial distance class. Pairwise kinship coefficients were regressed on the logarithm of spatial distance to estimate a regression slope (*blog*).

For each population, spatial locations were randomly permuted among individuals 10 000 times in order to test, for each spatial distance class, whether the observed mean kinship values were different from those expected under a random distribution of genotypes. To test the significance of the observed SGS pattern, a distribution of regression slopes was also constructed using a permutation test, and P values for the observed regression were estimated as the fraction of this distribution greater than the observed slope (tests available in SPAGEDI). Ramet- and genet-level analyses are detailed below.

Ramet-level analysis

Coupled to the traditional ramet-level analyses an additional method was performed using all sampled ramets but now considering only the kinship values for pairs between different genets (using the option 4.3.3.5.3, in SPAGEDI software, where categories corresponded to different clones). In this analysis all spatial information for a given multi-sampled genet is kept since all repetitions from a given genotype are used, but the potential inflating effect on SGS produced by clone mate pairs is removed. Hereafter we will refer to this method as the among-genet analyses. Where clonal growth results in the spatial clustering of

clone mates, the 'ramet level' will produce higher kinship values than the 'among genet' within the spatial range where clone mates are clumped, beyond which both take similar values. In fact, if we plot both correlograms together, we show that the point where the two curves merge is an estimate of the spatial range at which clonality has non-negligible effects on the SGS, here defined as clonal subrange. In order to further illustrate the clonal subrange we determined the probability of clonal identity (F_r) as a function of spatial distance (Harada & Iwasa 1996). For that purpose we computed, for a set of distance intervals, the fraction of pairs of ramets sharing the same multilocus genotype. The values were plotted on top of the above-described autocorrelogram. This analysis was performed using an R 1.6.1 (The R Development Core Team, 2002) code.

Genet-level analysis

SGS was characterized after removing clonal replicates from the data set and considering the central coordinates of each clone (average of *x* and *y* coordinates of clone mates). Using a genet's central coordinates for its spatial representation can be justified as this point is the most parsimonious position of the clone's birthplace. However this assumes isotropic growth and no disturbance causing loss of a sector of the clone. The first assumption is not supported by available information that shows that the origin of the patch/clone is always displaced relative to the geometric centre (Duarte & Sand-Jensen 1990; Vidondo *et al*. 1997). A resampling technique was used to analyse the variance in the estimates resulting from the selection of different spatial coordinates to represent the clone. A random representative ramet from each genotype repeated in the sample was resampled to create a matrix with a single copy of each genet, the procedure was repeated 100 times to estimate the dispersion of the estimates. The proportion of these 100 data sets yielding significant mean kinship values for the first distance class and/or significant regression slopes was recorded.

Indirect estimation of dispersal, and neighbourhood size

The intersection between the correlogram curve and the *x*-axis of the plot is often considered as an estimate of the distance within which individuals reproduce with their close relatives, or the radius of a patch (Epperson 2003). However, this method is highly dependent on the spatial scale of sampling (Fenster *et al*. 2003). Thus, we rather estimated the neighbourhood size (*Nb*), the number of individuals that characterize the strength of genetic drift in the population (Vekemans & Hardy 2004). A redefinition of the concept is proposed by Fenster *et al*. (2003), defining neighbourhood area as a circular area containing such *Nb* individuals, within which biparental inbreeding remains insignificant. If the SGS pattern is produced by an isolation-by-distance process, at drift–dispersal equilibrium in a two-dimensional space, *Nb* can be estimated from *blog* and is equal to $-(1 - F_{(1)})/blog$ (Hardy & Vekemans 1999; Fenster *et al*. 2003), where F_1 is the average F_{ij} between individuals belonging to the first distance class (here $F_1 = F_{[2m]}$). However, under the above-mentioned conditions, kinship is expected to decrease linearly with the logarithm of spatial distance for a restricted range (σ to 20σ, where σ is the axial standard deviation of gene dispersal distances; Rousset 1997). As σ is unknown, an iterative approach available in SPAGEDI was used to estimate *blog* using the observed genotype density as the effective population density (*D*). The neighbourhood area was calculated as the surface that would contain the *Nb* individuals for each population. Because *blog* depends to some extent on the sampling scale used and it is negative, we also estimated the *Sp* statistic which absolute value reflects the rate of decrease of pairwise kinship with distance (Vekemans & Hardy 2004). This allowed us to compare the SGS pattern in *C. nodosa* with the strength of patterns observed among other species. The *Sp* statistic is equal to $-blog/(1 - F_{(1)})$.

Finally we evaluated the relative importance of clonal growth and sexual reproduction to gene dispersal. First we computed the axial variance of gene dispersal mediated by clonal growth σ^2_{veg} (Gliddon *et al*. 1987), as one-half the mean squared distance between a ramet and the central coordinates of the clone to which it belongs. To calculate σ^2_{veg}, we used all sampled genets, not only those that had more than one ramet sampled, and in order to do so an arbitrary distance of 1 m (half the minimum distance between consecutive sample units) was considered for genets which appeared only once in the sample. Then we applied the Gliddon *et al*. (1987) model of parent–offspring dispersal variance (σ^2):

$$\sigma^2 = \frac{1}{2}\sigma^2_p + \sigma^2_s + \sigma^2_{veg} \Leftrightarrow \sigma^2 = \sigma^2_{sex} + \sigma^2_{veg} \qquad \text{(eqn 2)}$$

where σ^2_p is the pollen dispersal variance and σ^2_s the seed dispersal variance and consequently the sexual mediated dispersal variance is $\sigma^2_{sex} = 1/2\sigma^2_p + \sigma^2_s$. The genet-level SGS-based estimate of *Nb* (see above) is also a function of the sexual and vegetative components of dispersal variance and can be used to estimate the total variance $\sigma^2 = Nb/4\pi D$. An estimate of σ^2_{sex} can then be obtained by subtracting from σ^2 the above-estimated σ^2_{veg}.

Scoring errors and/or somatic mutations effects on SGS

We evaluated the potential bias caused by scoring errors or somatic mutations, on the strength of positive autocorrelation

at smaller distance classes. This problem can arise if clone mates are erroneously assigned as different, thus being included in the analyses where clonal repeats are excluded. We produced an R 1.6.1 (The R Development Core Team, 2002) routine to detect which pairs differ by only a single allele while being neighbours in the 2-m sampling scale. When such pairs were found the smaller genet was removed from the data set as a potential source of error. A total of 18 genotypes in Cadiz and 14 genotypes in Alfacs were removed. Separate analysis after the exclusion of these genotypes did not differ from those with the complete data set.

Results

Genotypic richness and clonal structure

The number of alleles amplified was 41 and 39, and a total of 83 and 95 different microsatellite multilocus genotypes (MLG) were identified for Cadiz Bay and Alfacs Bay, respectively. All repetitions of the same MLG, for all MLGs observed in more than one sample unit, had P_{sex} values < 0.05 and so were considered to result from repeated sampling of the same clone. Therefore, the number of different MLGs found corresponds to the number of clones present in our samples. The spatial organization of clonal repetitions (Fig. 2) shows that both meadows were dominated by a few large clones, which appeared aggregated in space. In Cadiz Bay and Alfacs Bay, respectively, only four and two clones had more than 10 repeats (representing 41% and 33% of the 220 sampled shoots) and only 24 and 18 clones appeared more than once (70% and 57% of the sampled shoots). This resulted in an extremely skewed distribution of clone size, as observed by the distribution of the linear dimension of the clones (Fig. 3), with a median clonal dimension of 3.6 m and 3.4 m in Cadiz Bay and Alfacs Bay, respectively. Genotypic richness was below 0.5 for both meadows, although Alfacs Bay showed higher levels (0.46) than Cadiz Bay (0.38). The highly skewed clone size distribution, and the low number of clones with more than one observation, indicate that this moderate genotypic richness is the result of the dominance of a few, large

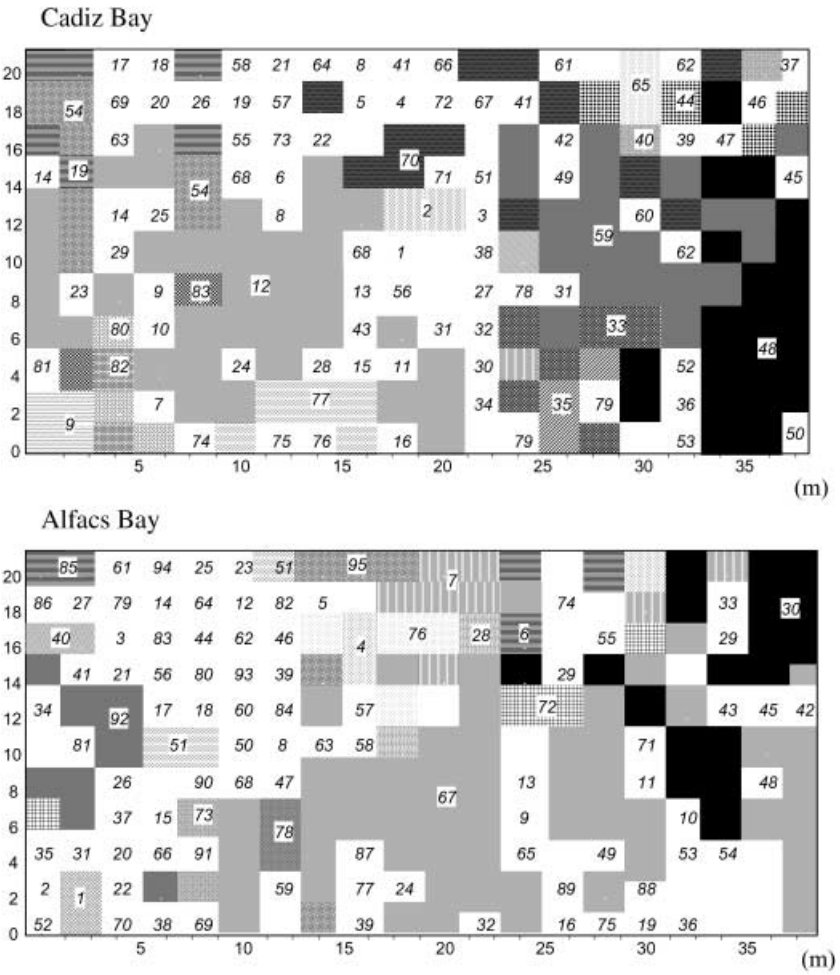

Fig. 2 Sampling grids of *Cymodocea nodosa* in Cadiz Bay and Alfacs Bay. The minimum distance between consecutive sampling points was 2 m. The numbers shown code the different genets; different patterns are used to represent each of the clones with more than one copy. Some sampling sites had no cover; these are represented by blank grid units.

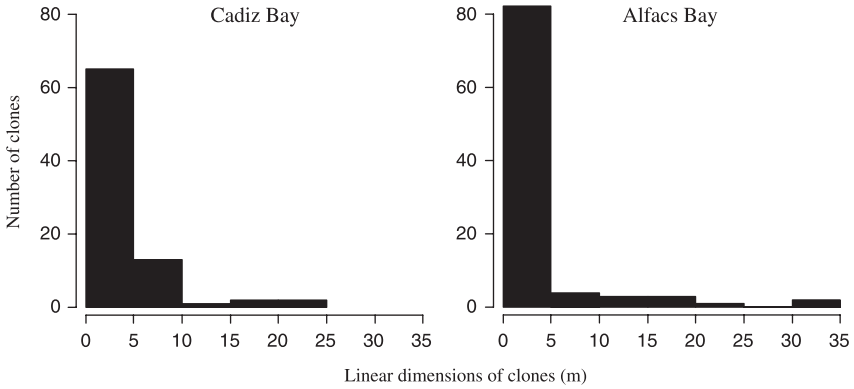

Fig. 3 Distribution of the linear dimensions of *Cymodocea nodosa* clones for Cadiz Bay and Alfacs Bay. The linear dimension is the minimum clone size estimated as the distance between the farthest clonemates.

Table 1 Number of alleles (*Na*), expected heterozygosity (H_E) and inbreeding coefficient (F_{IS}) for genet-level analysis and ramet-level analysis of *Cymodocea nodosa* at Cadiz Bay and Alfacs Bay. Significant departures from the null hypothesis of Hardy–Weinberg equilibrium are coded: ***$P < 0.001$; **$0.001 < P < 0.01$; and *$0.01 < P < 0.05$

	Cadiz Bay					Alfacs Bay			
	Genet level		Ramet level			Genet level		Ramet level	
Loci	*Na*	H_E	F_{IS}	H_E	F_{IS}	*Na*	H_E	F_{IS}	H_E	F_{IS}
Cn2–86	4	0.748	−0.220***	0.736	−0.281***	6	0.748	−0.022**	0.730	−0.172***
Cn2–38	5	0.611	−0.046	0.572	−0.101*	4	0.485	−0.016	0.486	−0.142
Cn2–14	3	0.498	−0.477***	0.482	−0.548***	4	0.617	0.015	0.576	−0.135**
Cn2–24	10	0.787	0.249***	0.819	0.152***	5	0.380	−0.187	0.451	−0.286***
Cn4–19	5	0.456	0.040	0.472	0.170***	6	0.652	0.005*	0.632	−0.166***
Cn2–16	3	0.523	−0.110	0.508	0.047	2	0.259	0.475***	0.174	0.350***
Cn2–18	3	0.447	−0.219	0.480	−0.331***	2	0.478	−0.162	0.478	−0.421***
Cn4–29	3	0.498	−0.357***	0.516	−0.356***	6	0.524	−0.120	0.496	−0.009***
Cn2–45	5	0.666	−0.191	0.664	−0.172***	4	0.662	−0.251***	0.646	−0.419***
Multilocus	41	0.582	−0.129***	0.583	−0.144***	39	0.534	−0.064***	0.519	−0.197***

clones, despite the presence of many unique clones in the samples.

Heterozygote excess

Both meadows showed significant heterozygote excesses before and after clonal replicates were removed (all $P < 0.001$) although the F_{IS} values were higher for the latter analysis (Table 1). We did not find any significant association between clone size and heterozygosity, $P = 0.58$ and $P = 0.07$ for Cadiz and Alfacs, respectively. When testing for genotypic linkage disequilibrium, and after applying Bonferroni correction, only nine (Cadiz) and six (Alfacs) pairs of loci, from a total of 36 pairs, rejected the null hypothesis of genotypes at one locus being independent from genotypes at the other locus. The pairs of loci involved were not consistent across the two sites.

Ramet-level analysis and clonal subrange

Clonal structure was characterized by analysing the spatial autocorrelation of microsatellite genotypes using the kinship coefficient (F_{ij}) at the ramet and among genet levels. The distance class where these correlograms merge (see Fig. 4) represents the clonal subrange, the distance range beyond which clonality has negligible effects on genetic structure, as less than 1% of the pairs are clonal. For Cadiz, the clonal subrange extended across shorter distances (20–25 m) than it did in Alfacs Bay (30–35 m). The probability of clonal identity (F_r), plotted on Fig. 4, declined with increasing distance, from around 25% for both meadows in the first distance class (2 m) to reach zero and 2.5% at 30 m in Cadiz and Alfacs, respectively. At the point where the ramet level and among genets level correlograms merge, F_r takes values lower than 1% (as less

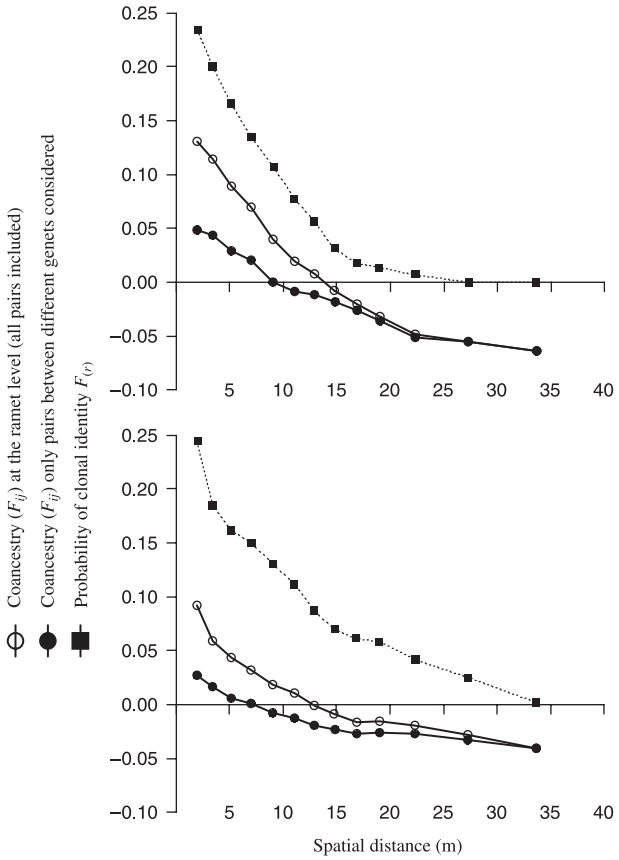

Fig. 4 Analysis of *Cymodocea nodosa* clonal structure by means of spatial autocorrelation analysis of kinship coefficients for Cadiz Bay and Alfacs Bay. Three distinct analyses were used: (i) a ramet-level analysis which includes all ramets sampled; (ii) an among-genet analysis, where only pairs between different genets are allowed (see Methods); and (iii) the probability of clonal identity. The spatial distance where the ramet-level and among-genet correlograms merge corresponds to a probability of clonal identity close to zero and estimates the radius of the clonal subrange.

than 1% of the pairs at that distance interval share the same genotype). These distances correspond to the dimensions of the largest clones found in each of the populations (Fig. 3).

Genet-level analysis and indirect dispersal estimates

A summary of results from the autocorrelation of genetic variation using kinship coefficients and different methods of data analysis is presented in Table 2. The coefficient estimates from the ramet and among genets levels are presented only for comparison with the genet-level estimates. Ramet-level estimates are higher due to the correlations between clone mates. When the central method was used to represent the spatial coordinates of the clones the average kinship coefficient was significantly positive at the first distance class for Cadiz ($F_{(2,m)} = 0.119$; $P < 0.001$) but not for Alfacs ($F_{(2,m)} = 0.021$; $P > 0.05$). Yet for the random selection method 100 and 68 of the 100 generated data sets had positive significant $F_{(2,m)}$ values for Cadiz [$F_{(2,m)} \pm$ SE (over 100 data sets) $= 0.089 \pm 9 \times 10^{-3}$] and Alfacs [$F_{(2,m)} \pm$ SE (over 100 data sets) $= 0.029 \pm 4 \times 10^{-3}$], respectively.

Within the sampled range, the average kinship coefficients between pairs of individuals declined linearly with the increasing logarithm of the spatial distance (Table 2) and there was a significant SGS pattern (all slope tests were significant, see Table 2 and Fig. 5). The steeper regression slopes (*blog*) for Cadiz Bay (−0.044; $P < 0.001$), than for Alfacs (−0.012 to −0.014; $P < 0.001$), resulted in smaller *Nb* estimates and a stronger *Sp* statistic in Cadiz (Table 2). The estimated vegetative component of gene dispersal σ^2_{veg} was 10.9 and 17.6 in Cadiz Bay and Alfacs Bay, respectively. The sexual component of dispersal (σ^2_{sex}) estimated on the basis of those values was consequently lower in Cadiz (7.1) than in Alfacs (20.5). Finally, the relative importance of the sexual and vegetative components of gene dispersal, described by the ratio $\sigma^2_{sex}/\sigma^2_{veg}$, was 0.65 in Cadiz and 1.16 in Alfacs.

Discussion

Spatial genetic structure and sexual reproduction

The pronounced SGS observed in Cadiz suggests extremely limited dispersal for *Cymodocea nodosa* in that bay. This pattern is the strongest observed so far for any seagrass species (Reusch *et al*. 1999a; Hämmerli & Reusch 2003b) and the *Sp* values found here are among the strongest patterns reported for land plants, observed in selfing herbaceous species (Caujapé-Castells & Pedrola-Monfort 1997; Bonin *et al*. 2001; review in Vekemans & Hardy 2004). Yet, under equal seed dispersal, dioecious species such as *C. nodosa* are expected to show lower SGS than selfing species, because of pollen flow (Vekemans & Hardy 2004). Seed dispersal in *C. nodosa* is expected to be limited as a consequence of seed size (Eriksson 1997; Inglis 2000), negative buoyancy, position at the base of the shoot buried in the sediment (Buia & Mazzella 1991), and association with female plants (Caye & Meinesz 1985). Nevertheless, the strong SGS observed here may also be related to limited pollen dispersal. Restricted pollen dispersal has been suggested from the observation that the abundance of seed production is related to the proximity of male to female plants in the seagrass beds (Caye & Meinesz 1985), although the authors did not quantify the spatial scale of this observation. Terrados (1993) did not detect pollen limitation, although this was only over distances of less than 0.5 m.

The weaker SGS pattern observed in Alfacs may be partially explained by the different disturbance regimes affecting both sites. The model used to estimate dispersal

Table 2 Summary of kinship autocorrelation in two *Cymodocea nodosa* meadows using different methods of analysing a data set from a clonal organism (see methods). Mean F_{ij} kinship values found for the shortest distance interval ($F_{(2,m)}$). The slope of the regression of mean kinship with the logarithm of spatial distance (*blog*) and the *Sp* statistic with the jackknife estimated standard error (*Sp*). Finally the estimated neighbourhood size (*Nb*) and the area containing such number of individuals based on the observed genet density in each meadow. *Nb* values for the genet-level (central) analysis are estimated using an iterative procedure (see methods). Significant values of $F_{(2,m)}$, *blog* and *Sp* are shown in bold ($\alpha = 0.025$). For the random method (last row) standard errors are given based on the distribution of parameters obtained after analysing 100 of these data sets

Method	$F_{(2,m)}$	*blog*	*Sp* (SE)	*Nb*	Area m² (radius m)
Cadiz Bay					
Ramet level	**0.133**	**−0.083**	**0.096** ± 0.022	10.4	101 (5.7)
Among gemet	**0.051**	**−0.049**	**0.052** ± 0.020	19.4	187 (7.7)
Genet level (central)	**0.119**	**−0.044**	**0.052** ± 0.016	23.5	226 (8.5)
Genet level (random)	$0.089 \pm 9 \times 10^{-3}$	$-0.044 \pm 1 \times 10^{-3}$	$0.048 \pm 2 \times 10^{-3}$	19.8 ± 0.8	191 ± 6 (7.8 ± 0.2)
Alfacs Bay					
Ramet level	**0.094**	**−0.044**	**0.050** ± 0.007	20.6	198 (7.9)
Among gemet	**0.030**	**−0.024**	**0.025** ± 0.008	40.4	390 (11.1)
Genet level (central)	0.021	**−0.014**	**0.015** ± 0.004	56.8	548 (13.2)
Genet level (random)	$0.029 \pm 4 \times 10^{-3}$	−0.012 ± 0.000	0.012 ± 0.000	80.9 ± 4.15	780 ± 40 (15.8 ± 0.5)

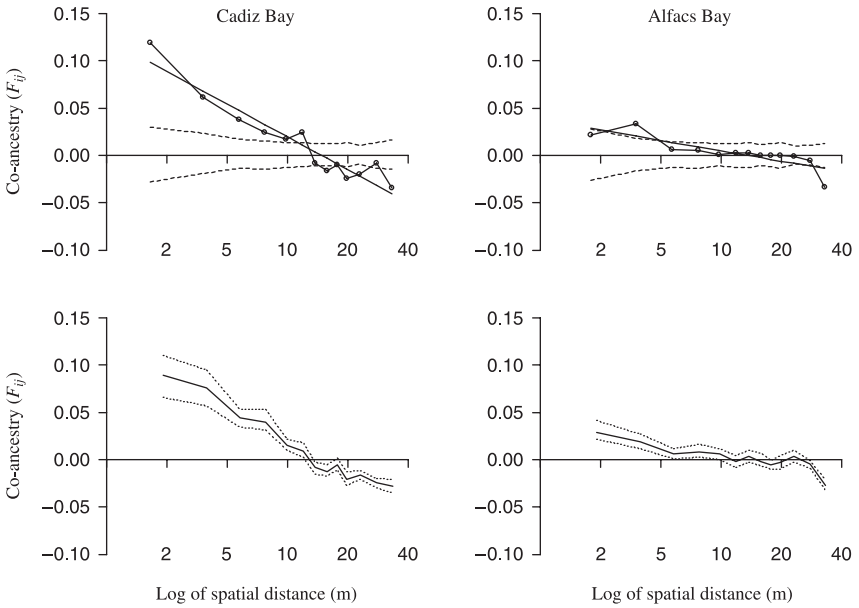

Fig. 5 Genet-level analysis correlogram showing mean kinship coefficients (circles) between individual *Cymodocea nodosa* genets as a function of spatial distance at Cadiz Bay and Alfacs Bay. The first row shows the correlogram produced when central coordinates were used to represent the spatial coordinates of the genet. Broken lines delimit 95% confidence intervals around the null hypothesis of random distribution of genets in space. In the second row the correlograms (continuous line) are based on the mean kinship coefficients found after 100 data files were analysed, each containing a single randomly selected ramet for each genotype. Dotted lines delimit the maximum and minimum values found. This procedure generates confidence intervals for the kinship and dispersal estimators.

assumes that the SGS has reached a stationary phase representative of the drift–dispersal equilibrium (Rousset 1997; Vekemans & Hardy 2004), whereas the periodical disturbance imposed by the migration of a subaqueous dune (Marbà *et al*. 1994; Marbà & Duarte 1995) can prevent the population from reaching the required equilibrium state. An SGS pattern takes several generations to develop depending on the scale of analyses. In Alfacs Bay this is by far longer than the disturbance period (Marbá *et al*. 1994; Marbá & Duarte 1995) suggesting that the SGS pattern observed could be a transient one. The analysis of the shape of the correlogram, at a spatial scale smaller than the axial standard deviation of gene dispersal distances (σ), can provide information about the relative contributions of seeds and pollen to the overall level of gene dispersal (Heuertz *et al*. 2003; Vekemans & Hardy 2004). In our case the initial curvature in Alfacs (downward concave form) suggests a more important contribution of seed dispersal than in Cadiz; this should be the most likely scenario if disturbance cleared space facilitating seed dispersal along the sediment surface. Another consequence of disturbance and higher population turnover should be a younger

meadow age. Both arguments could explain a weak SGS pattern, still influenced by founder events, such as observed here for Alfacs Bay.

Although a few genets dominated the studied meadows, the majority of the genets, in both sites, appeared only once. This high frequency of young genets suggests that both meadows are characterized by successful sexual reproduction and low probability of young genets to grow to older/larger clones. However, genotypic richness (*R*), which provides an estimation of the balance of sexual and clonal reproduction over several generations, was only moderate due to the presence of a few dominant clones, although equivalent to what has been found for other clonal plants (Ellstrand & Roose 1987). Nevertheless our results clearly suggest that, for the analysed meadows, sexual reproduction is an important means of population recruitment for *C. nodosa*. It is important, however, to keep in mind that seagrass populations can show a wide range of genotypic richness levels, from monoclonal stands (e.g. Waycott *et al*. 1996; Reusch *et al*. 1999b; Alberto *et al*. 2001; Billingham *et al*. 2003) to highly diverse ones (e.g. Procaccini & Mazzella 1996; Reusch *et al*. 2000; Coyer *et al*. 2004; Arnaud-Haond *et al*. 2005).

Considered together our findings of restricted dispersal, successful sexual recruitment, and skewed genet size distribution suggest that sexual recruitment in *C. nodosa* is more important at the local meadow scale than on an inter-meadow scale. This type of life history trait has been referred to as repeated seedling recruitment (RSR in Eriksson 1993) and has been reported for other plant species (Auge & Brandl 1997; Suzuki *et al*. 1999; Stehlik & Holderegger 2000; Auge *et al*. 2001; Shimizu *et al*. 2002; Ziegenhagen *et al*. 2003). Seeds lacking specialized dispersal traits are expected to exhibit a greater competitive ability than seeds with higher dispersal capacity (Eriksson 1997), as they may undergo selection resulting from intraspecific competition in a crowded environment. If the population shows some level of biparental inbreeding, the most homozygous seedlings might be affected by some level of inbreeding depression influencing the growth traits relevant for space competition in dense seagrass stands. Evidence for inbreeding depression affecting clonal growth has been reported for the seagrass *Zostera marina* (Hämmerli & Reusch 2003a). Such processes may partly explain the observed heterozygosity excess in both meadows, which persisted even when the clonal replicates were removed from the data set (Table 1). The initial seedling growth stage corresponds to the most important bottleneck for clonal patch development, characterized by high seedling mortality (80–90% annually, Duarte & Sand-Jensen 1990, 1996) and where only half of the clone patches survive longer than 0.8 year (Vidondo *et al*. 1997). Even though we did not find any association between clone size and heterozygosity, our sampling did not cover the complete size spectra of *C. nodosa* clones. It is thus possible that significant relationships between individual heterozygosity and the capacity to initiate patch growth would be observed had we sampled seedlings in the initial growth stages. Such a selection hypothesis awaits support by further investigation, but it is interesting to note that we have not observed heterozygote excess in young seedlings (less than 1 year old) from Cadiz Bay, analysed with the same microsatellite markers (Alberto *et al*. 2003).

Clonal structure and subrange

The spatial organization of *C. nodosa* clones found here (Fig. 2) reveals that clonal replicates tend to aggregate in space. Recently, Sintes *et al*. (2004) used a clonal growth model to follow the colonization of space by a single developing *C. nodosa* clone. These authors showed that the growing network is characterized by a guerrilla-type growth in the initial colonization phase, but later on, and merely due to simple clonal growth rules (rhizome elongation, rhizome branching rate, branching angle and spacer length between consecutive shoots), the clone becomes compacted and circular (4–5 years of age) and changes to a growth model typical of compact structures. The aggregation of clonal replicates found for the older/larger clones in this study validates the predictions of the Sintes *et al*. (2004) model for natural meadows composed of several growing clones, although most genets found here are still small clones in the early growth phases. This highly skewed clone size distribution indicates again high mortality at the early growth phases, a type of structure often found for clonal plants (Chung & Epperson 1999, 2000; Herben *et al*. 2002). At the periphery of larger clones there were smaller, apparently fragmented, groups of additional clonal replicates, perhaps produced by the decay of the rhizome connections. It is likely that through this process older compacted clones give rise to smaller clonally integrated units, independent of the older/larger original clone. Alternatively the apparent fragmentation of large clones could simply be due to clone intermingling, as in this study the spatial scale is not totally suitable to disentangle genetic diversity at scales smaller than 2 m.

We have here implemented an original method based on spatial autocorrelation to study the effects of clonality on the SGS pattern. This approach was based on pairwise kinship coefficients estimated for the data set containing all sampled individuals, analysed in two different ways; considering all possible pairs and only the among-genet pairs. We show that the point where the two resulting correlograms merge (Fig. 4) is equivalent to a probability of clonal identity (probability of sampling the same clone at a certain spatial distance) close to zero, termed the clonal subrange. Such characterization of the clonal subrange can also be used to select a minimum distance between adjacent

samples when the objective is to maximize the genotypic richness in a given sample or to estimate parameters that require removal of clonal replicates. For the meadows analysed, the probability of sampling the same clone declined to zero at 30 m in Cadiz whereas in Alfacs it was still 2.5% at that distance.

Indirect estimation of dispersal

Neighbourhood size (*Nb*) estimates indicate the balance between drift and gene flow at a local scale (Fenster *et al*. 2003). Smaller *Nb* estimates, around 20–24 in Cadiz, suggest that genetic drift and inbreeding play an important role, and that the homogenizing effects of gene flow should be low. In order to estimate the neighbourhood area we should have used the effective density (*D*) instead of the observed genotype density (Hardy & Vekemans 2002). However, the former is extremely difficult to derive for clonal plants, where the simple estimation of the number of individuals in a population is a challenge (distinction between genets and ramets). By using the observed genotype density to estimate patch size we are most likely underestimating the total number of genotypes (*G*) in the surface analysed, because *G* is a function of the sampling effort. On the other hand, and partially compensating for this, the real effective population size is expected to be smaller than *G* (Orive 1993), an effect which in this case is magnified by the unbalanced individual contribution to reproduction expected from the skewed distribution of clone sizes.

We estimated the axial variance of the clone mates' spatial distribution (σ^2_{veg}) as an alternative way to quantify the contribution of clonal growth to gene dispersal. The model of Gliddon *et al*. (1987) and the *Nb* value estimated through the SGS analysis can be used to extract the sexual component, allowing estimation of the relative importance of sexual vs. vegetative dispersal. The results obtained suggest that, at least for Cadiz Bay, clonal spread might be an important gene dispersal vector, equivalent to sexual reproduction. However the comparison is based on assumptions made concerning the effective density and caution must be exercised in its interpretation. Also Gliddon *et al*.'s formula does not assume overlapping generations and the regression slope gives a mean estimate of dispersal over several generations, whereas the axial variance estimated from the width of genets gives the current vegetative dispersal. Finally, vegetative dispersal may be underestimated because of edge effects associated with the sampling scale. Ecological and genetic simulation studies such as the ones produced by Sintes *et al*. (2004) and Heuertz *et al*. (2003) should be employed together in order to validate the methods presented here and/or provide additional ways of describing the influence of clonal reproduction on SGS.

Acknowledgements

This research is a contribution of the EU-project M&MS (ref. EVK3-CT-2000-00044), and project PNAT/1999/BIA/15003/C of the Portuguese Science Foundation (FCT). We thank T. Simões, J.J. Vergara and F. Brun for help in field collections and L. Correia for technical help, C. Perrin and G. Pearson for a careful reading of this manuscript and anonymous reviewers for their suggestions. Fellowships to F. Alberto (PhD) and S. Arnaud-Haond (postdoctoral) were granted by FCT and the European Social Fund.

References

Alberto F, Mata L, Santos R (2001) Genetic homogeneity in the seagrass *Cymodocea nodosa* at its northern Atlantic limit revealed through RAPD. *Marine Ecology Progress Series*, **221**, 299–301.

Alberto F, Correia L, Billot C, Duarte CM, Serrão E (2003) Isolation and characterization of microsatellite markers for the seagrass *Cymodocea nodosa*. *Molecular Ecology Notes*, **3**, 397–399.

Arnaud-Haond S, Alberto F, Procaccini G, Serrão EA, Duarte CM (2005) Assessing genetic diversity in clonal organisms: low diversity or low resolution? Combining power and cost-efficiency in selecting markers. *Journal of Heredity*, **3**, 1–7.

Auge H, Brandl R (1997) Seedling recruitment in the invasive clonal shrub, *Mahonia aquifolium* Pursh (Nutt). *Oecologia*, **110**, 205–211.

Auge H, Neuffer B, Erlinghagen F, Grupe R, Brandl R (2001) Demographic and random amplified polymorphic DNA analyses reveal high levels of genetic diversity in a clonal violet. *Molecular Ecology*, **10**, 1811–1819.

Billingham M, Reusch TB, Alberto F, Serrão EA (2003) Is asexual reproduction more important at geographical limits? A genetic test of the seagrass *Zostera marina* in the Ria Formosa, Portugal. *Marine Ecology Progress Series*, **265**, 77–83.

Bonin I, Ronfort J, Wozniak F, Olivierri I (2001) Spatial effects and rare outcrossing events in *Medicago truncatula* (Fabaceae). *Molecular Ecology*, **10**, 1371–1384.

Buia MC, Mazzella L (1991) Reproductive phenology of the Mediterranean seagrasses *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa* (Ucria) Aschers, and *Zostera noltii* Hornem. *Aquatic Botany*, **40**, 343–362.

Caujapé-Castells J, Pedrola-Monfort J (1997) Space-time patterns of genetic structure within a stand of *Androcymbium gramineum* (Cav.) McBride (Colchicaceae). *Heredity*, **79**, 341–349.

Caye G, Meinesz A (1985) Observations of the vegetative development, flowering and seeding of *Cymodocea nodosa* (Ucria) Acherson on the Mediterranean coasts of France. *Aquatic Botany*, **22**, 277–289.

Chung MG, Epperson BK (1999) Spatial genetic structure and clonal and sexual reproduction in populations of *Adenophora grandiflora* (Campanulaceae). *Evolution*, **53**, 1068–1078.

Chung MG, Epperson BK (2000) Clonal and spatial genetic structure in *Eurya emarginata* (Theaceae). *Heredity*, **84**, 170–177.

Chung MG, Chung JM, Chung MY, Epperson BK (2000) Spatial distribution of allozyme polymorphisms following clonal and sexual reproduction in populations of *Rhus javanica* (Anacardiaceae). *Silvae Genetica*, **48**, 223–227.

Coyer JA, Diekmann OE, Serrao EA *et al*. (2004) Genetic differentiation of the seagrass, *Zostera noltii*, populations throughout its european, asian and african range. *Marine Ecology Progress Series*, **281**, 51–62.

Dorken ME, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *Journal of Ecology*, **89**, 339–350.

Doyle JJ, Doyle JL (1988) Natural interspecific hybridization in eastern North American Claytonia. *American Journal of Botany*, **75**, 1238–1246.

Duarte CM, Sand-Jensen K (1990) Seagrass colonization: patch formation and patch growth in *Cymodocea nodosa*. *Marine Ecology Progress Series*, **65**, 193–200.

Duarte CM, Sand-Jensen K (1996) Nutrient constraints on establishment from seed and on vegetative expansion of the Mediterranean seagrass *Cymodocea nodosa*. *Aquatic Botany*, **54**, 279–286.

Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant species. *American Journal of Botany*, **74**, 123–131.

Epperson BK (2003) *Geographical Genetics: Monographs in Population Biology*. Princeton University Press, Princeton, New Jersey.

Eriksson O (1993) Dynamics of genets in clonal plants. *Trends in Ecology & Evolution*, **8**, 313–316.

Eriksson O (1997) Clonal life histories and the evolution of seed recruitment. In: *The Ecology and Evolution of Clonal Plants* (eds de Kroon H, van Groenendael J), pp. 211–226. Backhuys Publishers, Leiden, The Netherlands.

Fenster CB, Vekemans X, Hardy OJ (2003) Quantifying gene flow from spatial genetic structure data in a metapopulation of *Chamaecrista fasciculate* (Leguminosae). *Evolution*, **57**, 995–1007.

Gliddon C, Belhassen E, Gouyon PH (1987) Genetic neighborhoods in plants with diverse systems of mating and different patterns of growth. *Heredity*, **59**, 29–32.

Hämmerli A, Reusch TBH (2003a) Inbreeding depression influences genet size distribution in a marine angiosperm. *Molecular Ecology*, **12**, 619–629.

Hämmerli A, Reusch TBH (2003b) Genetic neighbourhood of clone structures in eelgrass meadows quantified by spatial autocorrelation of microsatellite markers. *Heredity*, **91**, 448–455.

Harada Y, Iwasa Y (1996) Analyses of spatial patterns and population processes of clonal plants. *Researches on Population Ecology*, **38**, 153–164.

Harada Y, Kawano S, Iwasa Y (1997) Probability of clonal identity: inferring the relative success of sexual versus clonal reproduction from spatial genetic patterns. *Journal of Ecology*, **85**, 591–600.

Hardy OJ, Vekemans X (1999) Isolation by distance in a continuous population: reconciliation between spatial autocorrelation and population genetics models. *Heredity*, **83**, 145–154.

Hardy OJ, Vekemans X (2002) SPAGEDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Hemminga MA, Duarte CM (2000) *Seagrass Ecology*. Cambridge University Press, Cambridge.

Herben T, Hara T, Handicova V *et al.* (2002) Neighborhood effects and genetic structure in a clonal grass: the role of the spatial structure of the environment. *Plant Species Biology*, **16**, 1–11.

Heuertz M, Vekemans X, Hausman F, Paladas M, Hardy OJ (2003) Estimating seed vs. pollen dispersal from spatial genetic structure in the common ash. *Molecular Ecology*, **12**, 2483–2495.

Inglis GJ (2000) Variation in the recruitment behaviour of seagrass seeds: implications for population dynamics and resource management. *Pacific Conservation Biology*, **5**, 251–259.

Loiselle BA, Sork VL, Nason JD, Graham C (1995) Spatial genetic structure of a tropical understory shrub. *American Journal of Botany*, **82**, 1420–1425.

Lovett Doust L (1981) Population dynamics and local specialization in a clonal perennial (*Rannunculus repens*) I. The dynamics of ramets in contrasting habitats. *Journal of Ecology*, **69**, 743–755.

Marbà N, Cebrian J, Enríquez S, Duarte CM (1994) Migration of large scale subaqueous bedforms measured using seagrasses (*Cymodocea nodosa*) as tracers. *Limnology and Oceanography*, **39**, 126–133.

Marbà N, Duarte CM (1995) Coupling of seagrass (*Cymodocea nodosa*) patch dynamics to subaqueous dune migration. *Journal of Ecology*, **83**, 381–389.

Marbà N, Duarte CM (1998) Rhizome enlongation and seagrass clonal growth. *Marine Ecology Progress Series*, **174**, 269–280.

Orive M (1993) Effective population size in organisms with complex life-histories. *Theoretical Population Biology*, **44**, 316–340.

Parks JC, Werth CR (1993) A Study of spatial features of clones in a population of Bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). *American Journal of Botany*, **80**, 537–544.

Procaccini G, Mazzella L (1996) *Genetic Variability and Reproduction in Two Mediterranean Seagrasses*. Seagrass Biology: Proceedings of an International Workshop, pp. 85–92. Rottnest Island, Western Australia.

Prugnolle F, Choisy M, Théron A, Durand P, De Meeûs T (2004) Sex-specific correlation between heterozygosity and clone size in the trematode *Schistosma mansoni*. *Molecular Ecology*, **13**, 2859–2864.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

R Development Core Team (2004) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3, URL http://www.R-project.org.

Reusch TBH, Hukriede W, Stam WT, Olsen JL (1999a) Differentiating between clonal growth and limited gene flow using spatial autocorrelation of microsatellites. *Heredity*, **83**, 120–126.

Reusch TBH, Boström C, Stam WT, Olsen JL (1999b) An ancient eelgrass clone in the Baltic Sea. *Marine Ecology Progress Series*, **183**, 301–304.

Reusch TBH, Stam WT, Olsen JL (2000) A microsatellite-based estimation of clonal diversity and population subdivision in *Zostera marina*, a marine flowering plant. *Molecular Ecology*, **9**, 127–140.

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Shimizu Y, Ando M, Sakai F (2002) Clonal structure of natural populations of *Cryptomeria japonica* growing at different positions on slopes, detected using RAPD markers. *Biochemical Systematics and Ecology*, **30**, 733–748.

Sintes T, Marbà N, Duarte CM, Kendrick GA (2004) Nonlinear processes in seagrass colonisation explained by simple clonal growth rules. *Oikos*, **108**, 165–175.

Stehlik I, Holderegger R (2000) Spatial genetic structure and clonal diversity of *Anemone nemorosa* in late successional deciduous woodlands of Central Europe. *Journal of Ecology*, **88**, 424–435.

Suzuki JI, Herben T, Krahulec F, Hara T (1999) Size and spatial pattern of *Festuca rubra* genets in a mountain grassland: its relevance to genet establishment and dynamics. *Journal of Ecology*, **87**, 942–954.

Terrados J (1993) Sexual reproduction and seed banks of *Cymodocea nodosa* (Ucria) Ascherson meadows on the southeast Mediterranean coast of Spain. *Aquatic Botany*, **46**, 293–299.

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, **13**, 921–935.

Vidondo B, Duarte CM, Middelboe AL, Stefasen K, Lutzen T, Nielsen SL (1997) Dynamics of a landscape mosaic: size and age distributions, growth and demography of seagrass *Cymodocea nodosa* patches. *Marine Ecology Progress Series*, **158**, 131–138.

Waycott M, Walker DI, James SH (1996) Genetic uniformity in *Amphibolis antarctica*, a dioecious seagrass. *Heredity*, **76**, 578–585.

Weir BS (1990) *Genetic Data Analysis*. Sinauer Associates, Sunderland, Massachusetts.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wright S (1977) *Evolution and the Genetics of Populations*. Vol. 4. *Variability Within and Among Populations*. University of Chicago Press, Chicago.

Ziegenhagen B, Bialozyt R, Kuhlenkamp V, Schulze I, Ulrich A, Wulf M (2003) Spatial patterns of maternal lineages and clones of *Galium odoratum* in a large ancient woodland: inferences about seedling recruitment. *Journal of Ecology*, **91**, 578–586.

Filipe Alberto conducted this study as part of his PhD, and is interested in population genetics of marine organisms. Licínia Gouveia collaborated in this study as part of her undergraduate studies in marine biology. Sophie Arnaud-Haond is a post doctoral associate interested in marine evolution and population genetics, currently focusing on marine plants and clonal organisms in general. José L. Pérez-Llorens is interested in ecophysiology of marine macrophytes. Carlos M. Duarte leads a team studying marine biodiversity from the genetic, species and habitat level to global biogeochemical cycles. Ester A. Serrão leads a research group that is primarily interested in marine ecology, adaptation and population genetics.
