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The interaction of environment and genetic diversity within meadows of the seagrass *Posidonia australis* (Posidoniaceae)

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ABSTRACT: Understanding the extent and impact of factors influencing the levels and structuring of genetic diversity within natural populations is a key objective of ecological genetics. For marine angiosperms, variation in abiotic environmental factors at the local scale can have a major influence on levels of clonality and spatial genetic structure, and thus influence mating systems, sexual reproduction, and recruitment. Identifying the key drivers of genetic structuring is critical for genetic management of ecological restoration success, especially in systems where the nature and extent of clonality is highly variable. Here, we quantify clonality and patterns of genetic structure in the temperate Australian seagrass *Posidonia australis*. We examine the location of meadows in relation to water movement and prevailing winds to assess their relative influence on local spatial genetic structuring. Measures of genetic diversity, assessed with 7 polymorphic microsatellite loci, were highly variable across 13 meadows sampled within and around a natural embayment on the west coast of Australia. The overall structure of *P. australis* meadows across this region is best explained as one of 'chaotic' genetic patchiness, with significant differentiation among most meadows (pairwise F_{ST} values), high levels of genetic diversity in meadows that are in more open waters, and lower genetic diversity at inshore sites facing strong prevailing winds at the time of seed dispersal or that have little water movement. A strong isolation by distance relationship within the embayment is consistent with prevailing winds (which create surface currents) at the time of peak pollen and seed release, strongly influencing dispersal direction.

KEY WORDS: Chaotic genetic patchiness · Isolation by distance · Microsatellite DNA · Windage · Inshore currents · Seagrass meadows

INTRODUCTION

Spatial genetic structure within and among natural populations is largely dependent on the interaction between life history traits, such as mating and dispersal, and the biotic and abiotic factors impacting on the expression of these traits. The effects of these factors tend to be more predictable in terrestrial systems, and fit population models better (Hellberg et al. 2002). In marine systems, apparent randomness or 'chaotic genetic patchiness' appears to be more typical (Johnson & Black 1984, Johnson et al. 1993, Becheler et al. 2010, Selkoe et al. 2010) as a consequence of stochastic connectivity due to more chaotic

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nearshore coastal circulations (e.g. Siegel et al. 2008). Consequently, the population is a difficult concept to apply in marine systems (Waples & Gaggiotti 2006, Becheler et al. 2010).

In addition, marine flowering plants grow in large, clonal meadows with variable spatial arrangement of genets. Like many terrestrial angiosperms, marine plants display a mixed mating strategy, in which sexual events (production of new seedlings) are combined with asexual reproduction (clonal or vegetative growth) (Honnay & Jacquemyn 2008). A recent review of reproduction in clonal plants (Silvertown 2008) suggests a pattern of new 'populations' establishing through sexual reproduction and expanding largely by asexual reproduction (akin to the initial seedling recruitment (ISR) strategy in Eriksson 1993). While ISR is likely to prevail in stable environments (Becheler et al. 2014), an alternative repeated seedling recruitment (RSR) strategy is expected in areas of frequent disturbance (Eriksson 1993). Thus, the chaotic genetic patchiness may be strongly influenced by these 2 strategies.

Seagrasses have long been regarded as essentially clonal (Kendrick et al. 2005). However, the different sampling methods and scales employed have made it difficult to compare studies assessing variation in clonal species and its drivers (Arnaud-Haond et al. 2007). Despite this, the general trend appears to be one in which local environmental conditions and geological history are the main factors determining genetic structure in seagrasses (Procaccini et al. 2001, Serra et al. 2010). If this is the case, then detailed studies characterising local marine environments are required to examine the effects of abiotic factors, such as wind speed and direction, wave action, tides, sedimentation rates, and local currents, on population growth, pollen and seed dispersal, and successful recruitment into established (dense versus patchy vegetation) and degraded meadows. However, few studies have examined the impacts of these factors on local genetic structure, where regional oceanography (circulation of water masses in shallow, inshore, coastal areas) are likely to strongly influence dispersal (Källström et al. 2008, van Dijk et al. 2009, Serra et al. 2010).

Seagrass meadows represent important, highly productive coastal ecosystems worldwide (Costanza et al. 1997, Orth et al. 2006). A decline in seagrass communities has been recorded in many parts of the world and attributed to a range of natural and anthropogenic impacts (Hemminga & Duarte 2000, Kendrick et al. 2002, Waycott et al. 2009). Restoration of seagrass meadows following their disturbance or removal from coastal marine environments is now a priority action globally (Orth et al. 2006). With the increase in reported catastrophic marine introductions (e.g. Bourdouresque & Verlaque 2002, Rilov & Crooks 2009), transfer of non-related genetic stock in a marine environment is not a recommended practice, yet we know very little about the population genetic structure and processes driving observed patterns in most seagrasses.

Considering the complete long-term failure of most marine restoration programmes (see van Katwijk et al. 2009, Cunha et al. 2012), programmes associated with *Posidonia australis* (Statton et al. 2012) suggest that detailed examinations of population genetic structure using more variable markers with standardized sampling protocols are required (see Arnaud-Haond et al. 2007). Combining genetic approaches with environmental and ecological approaches provides a better understanding of the important links between oceanography, ecology, and gene flow (Selkoe et al. 2010, White et al. 2010).

Recent developments in molecular methods and spatial modelling tools have improved our ability to detect and describe fine-scale genetic patterns in clonal species, with higher levels of genetic diversity and connectivity observed than previously appreciated (e.g. Reusch 2002, van Dijk et al. 2009, Bricker et al. 2011). This suggests that sexual reproduction, which increases genetic diversity due to genetic recombination, makes an extremely important contribution to seagrass ecology. We focused on *P. australis*, a species that has been highly impacted around major human population centres (e.g. Kendrick et al. 2002) and subsequently targeted for restoration (Bastyan & Cambridge 2008, Verduin et al. 2012, Sinclair et al. 2013, Statton et al. 2013). We identified multilocus genotypes (MLGs), using 7 microsatellite loci, within and among selected meadows to estimate clonal diversity and population genetic parameters. This study provides new insight into the population genetic structure within *P. australis* meadows and the interaction with abiotic conditions at a local scale.

MATERIALS AND METHODS

Study species

Posidonia australis is a slow-growing, perennial species, with a widespread distribution occurring from Shark Bay in northern Western Australia to Wallis Lake in central New South Wales, and along

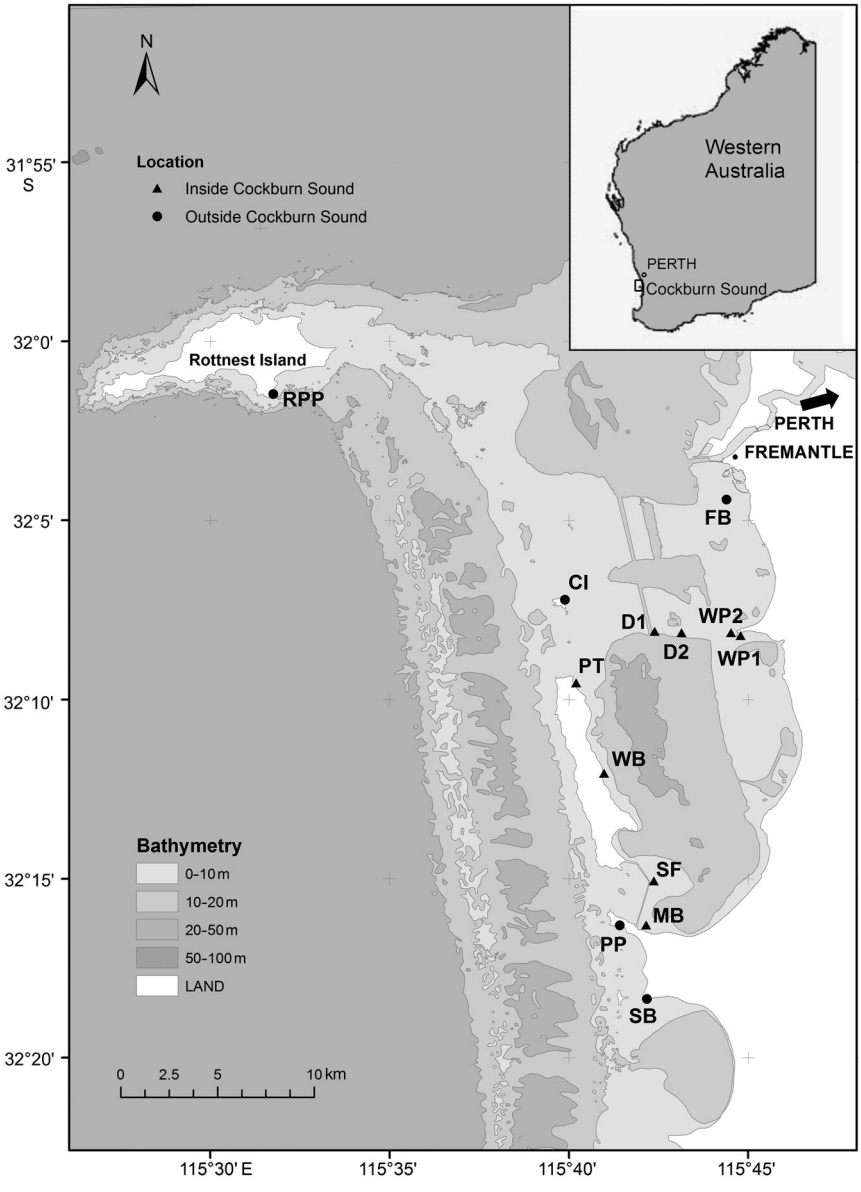

Fig. 1. Map showing the 13 sampled *Posidonia australis* meadows from the Cockburn Sound area, Western Australia. Sample site codes are: RPP = Parker Point, Rottnest Island, FB = Fishing Boat Harbour, CI = Carnac Island, D1 = Parmelia Bank 1 (west), D2 = Parmelia Bank 2 (east), WP1 = Woodman Point 1, WP2 = Woodman Point 2, PT = Pig Trough Bay, Garden Island, WB = Walking Beach, Garden Island, SF = Southern Flats, MB = Mangles Bay, PP = Point Peron, SB = Safety Bay. Note that the thin line between MB and SF is a man-made road and bridge access to Garden Island. Black triangles indicate the 8 meadows included within Cockburn Sound

the northern coast of Tasmania including Bass Strait Islands. It grows in large continuous meadows in 1 to 15 m of water, favouring the more sheltered bays and reef enclosures (Carruthers et al. 2007). Like all seagrasses, *P. australis* is a flowering plant adapted to a submerged life (den Hartog 1970). Peak flowering occurs in winter (July and August) and is likely triggered by cooler water temperature and day length, with seed release occurring in late November (early summer) (Cambridge & Hocking 1997).

Field site descriptions and meadow sampling

Posidonia australis was sampled from along a 40 km length of coastline on the west coast of Western Australia, in Perth metropolitan waters (Fig. 1). Sampling focused on Cockburn Sound, a natural embayment ~16 km long and 7 km wide. The embayment is protected by Garden Island and a reef system to the west that provides shelter from the main oceanic currents and winter storms. The seagrass meadow area within Cockburn Sound has declined significantly (~77%), largely due to the effects of eutrophication, industrial development, and sand-mining (Kendrick et al. 2002). Meadows immediately to the north are largely stable or have increased in area (Kendrick et al. 2000, 2008).

Nearshore coastal waters in south-western Australia are influenced by a high-energy wave regime generated by the fetch of winds from the south-west (Collins 1988). Within Cockburn Sound, wave energy is significantly reduced and consists primarily of low amplitude 'wind chop' with maximum-recorded wave heights of ~1 m (Department of Transport pers. comm.). Diurnal land and sea breezes dominate in summer when *P. australis* fruit(s) are released, with relatively light southeasterly winds in the evenings and early mornings and strong, persistent southwesterly winds in the afternoons (Ruiz-Montoya et al. 2012). Sea conditions that develop during summer thus respond to this wind pattern and are largely from the southwest. In winter, when pollen is released, prevailing winds are more variable, with the strongest storm winds coming from the northwest and southwest, generating mainly westerly and

Table 1. Sampling locations for *Posidonia australis* meadows. Sample area and interval between shoots have been standardized for direct comparisons of clonal diversity. * = the 8 meadows included within Cockburn Sound. The mean pairwise distance between shoots is the mean distance between all pairs of sampled shoots within a meadow. Site classifications based on Valesini et al. (2003). Pop. = population; MPD = mean pairwise distance between shoots

Pop.	Sample location	Abbr.	Latitude (S)	Longitude (E)	Year sampled	Sampling method	MPD (m)	Depth (m)	Site classification	Exposure	Dist. to nearest shore (m)
1	Parker Point, Rottnest Island	RPP	32° 1.470′	115° 31.760′	2009	Random coordinates	17.1	2.5	Highly sheltered (1)	S, SE	76
2	Fishing Boat Harbour	FB	32° 4.416′	115° 44.399′	2009	Random coordinates	17.7	3.8	Moderately exposed (4)	W, SW, S	1024
3	Carnac Island	CI	32° 7.200′	115° 39.000′	2009	Random coordinates	17.4	1.1	Highly sheltered (1)	E	55
4*	Parmelia Bank 1 (west)	D1	32° 8.096′	115° 42.388′	2004	1.0 m interval	10.1	6.1	Moderately sheltered (2)	NE, N, W, S, SE	3591
5*	Parmelia Bank 2 (east)	D2	32° 8.130′	115° 43.140′	2004	5.0 m interval	20.3	5.0	Moderately sheltered (2)	NE, N, W, S, SE	2413
6*	Woodman Point 1	WP1	32° 8.176′	115° 44.774′	2004	2.0 m interval	16.2	1.4	Moderately sheltered (2)	S	45
7*	Woodman Point 2	WP2	32° 8.130′	115° 44.524′	2004	2.0 m interval	16.2	2.1	Moderately sheltered (2)	W, SW, S	241
8*	Pig Trough Bay, Garden Island	PT	32° 9.530′	115° 40.205′	2010	random coord	19.9	0.8	Highly sheltered (1)	E	163
9*	Walking Beach, Garden Island	WB	32° 12.055′	115° 40.986′	2010	>2.0 m interval	34.0	0.6	Highly sheltered (1)	E	20
10*	Southern Flats	SF	32° 15.064′	115° 42.374′	2009	Random coordinates	20.0	2.2	Highly sheltered (1)	N, E, S	1491
11*	Mangles Bay	MB	32° 16.298′	115° 42.158′	2009	Random coordinates	20.0	1.2	Highly sheltered (1)	NE	290
12	Point Peron	PP	32° 16.333′	115° 41.510′	2010	>2.0 m interval	17.6	1.0	Moderately exposed (4)	S, SW	30
13	Safety Bay	SB	32° 18.319′	115° 42.227′	2004	0.5 to 1.5 m interval	17.2	2.0	Highly sheltered (1)	SW, S, SE	50

southwesterly seas. Tides are micro-tidal and predominantly diurnal, with a maximum spring tidal range in the order of 0.9 m (mean <0.5 m, Hearn 1991). Water exchange between Cockburn Sound and the open ocean is restricted by Parmelia Bank to the north, and a narrow channel between Point Peron and Garden Island to the south (Fig. 1).

To assess the possible influences of local conditions on the genetic structure of established seagrass meadows, we determined the position of each meadow in relation to prevailing winds (and wave action) in July (winter) and November (early summer), which coincide with peaks in potential gene flow through pollen and seed dispersal. Our sampling sites were designated to 3 of the 6 inshore habitats classified by Valesini et al. (2003): highly sheltered from wave activity (1), moderately sheltered from wave activity (2), and moderately exposed to wave activity (4) (Table 1). In addition, we recorded depth, distance from the nearest shore, and direction of exposure for each site. This covers the subset of local environmental variables, which gave the best correlation for discriminating between 6 *a priori* habitat types (Valesini et al. 2003). All our sites were inshore on flat or gently sloping gradients, with the exception of the Parmelia Bank sites (D1 and D2), which were in deeper, more open water.

We collected 50 individual *P. australis* shoots from 13 meadows, including all large remaining meadows within and around Cockburn Sound (Fig. 1, Table 1). Shallow sites were sampled by wading, while deeper sites were sampled on SCUBA. Initial sampling at different distance intervals (in 2004, Table 1) was used to assess the extent of individual clones as well as determine the optimum distance interval to obtain a representative sample of genetic diversity at each site. We subsequently followed guidelines for standardizing sampling of clonal organisms (Arnaud-Haond et al. 2007), sampling from 50 randomly generated coordinates within a 50 m diameter, except at the Garden Island site (WB) where the meadow was extremely narrow and linear, in which case we used a minimum 2 m interval, as determined by the 2004 sampling trial. Precise GPS locations were recorded for each shoot sample. The 2004 samples were all dried and stored in silica gel prior to DNA extraction. The 2009–2010 samples were fresh frozen to improve DNA quality: the green leaves were removed to reveal the smooth leaf meristem. The meristem was then cut longitudinally into 2 pieces (a and b samples) and frozen at −80°C prior to DNA extraction.

DNA extraction and genotyping

DNA was extracted using a modified polyvinyl pyrrolidone–sodium dodecyl sulphate method (Waycott & Barnes 2001, Sinclair et al. 2009). Seven polymorphic microsatellite loci (*Pa*A1, *Pa*A105, *Pa*A120, *Pa*B6, *Pa*B8, *Pa*B112, *Pa*D113) were amplified using PCR conditions described in Sinclair et al. (2009). This represents a subset of 10 loci characterised in *P. australis*, but contains only those loci known to be polymorphic at this sampling scale. PCR products were pooled where possible prior to genotyping on a CEQ 8800 Genetic Analysis System (Beckman Coulter). Allele sizes were determined using size standard 400 and scored with the aid of Beckman software. Prior to analysis, the data set was assessed for the presence of null alleles, stuttering, or large allele dropout with Micro-Checker (van Oosterhout et al. 2004).

Clonal structure and genetic diversity within meadows

Estimates of within-meadow genetic diversity are confounded in clonal species as clones vary in their size, age, and with different sampling schemes (different distances between sampled shoots within a seagrass meadow). The same MLG may result from re-sampling the same clone (due to vegetative growth where different ramets of the same individual are sampled), different individuals having the same recombination events, and the possible occurrence of somatic mutations or scoring errors. The probability that the identical MLG originated from a different (sexually) reproductive event (p_{sex}) was assessed (Arnaud-Haond et al. 2007). MLGs were considered as belonging to the same multilocus lineage (MLL) when they differed by a single allele. However, due to the somewhat subjective nature of defining MLLs (i.e. when a single MLG differs from multiple MLGs by one allele at different loci), and that we have high confidence in scoring of genotypes at these loci, based on a mating system study in neighbouring meadows (Sinclair et al. 2014), we have based all our diversity estimates on MLGs.

Clonal diversity was assessed by estimating 4 metrics using the software GenClone 2.1 (Arnaud-Haond & Belkhir 2007). These metrics were clonal richness ($R = (G - 1)/(N - 1)$, where G = number of MLGs and N = number of samples), Simpson's diversity index D^* (clonal heterogeneity) where $D^* = 1 - \sum\pi^2$, where π is the frequency of the MLG detected in the sample, clonal evenness ED^*, and slope of Pareto distribution β (clonal distribution) as measures of clonal heterogeneity among meadows (Arnaud-Haond et al. 2007). The Pareto distribution is used as a continuous approximation of the cumulative frequency of the genets composed of a particular number of ramets. The parameter β is derived as the slope of the fitted log–log regression equation, describing the rate of decline in the relative frequency of ramets that belong to a MLG of size equal to or larger than a given number of ramets. The Pareto index β takes a value ranging from 0 to infinity (∞). A high evenness of clonal lineages all having approximately comparable sizes will result in a steep slope (high β), whereas a skewed distribution with very few large clonal lineages will result in a shallow slope (low β). These methods allow for a better comparison of clonality estimates across meadows with different sampling regimes. To determine the effect of sampling method on genetic diversity indices, we calculated the mean pairwise distance between samples within each meadow, and plotted this against R.

Genetic diversity was assessed within each sampled meadow using GENALEX 6.5 (Peakall & Smouse 2012). Genetic diversity within meadows was estimated as the total number of alleles (N_a), number of private alleles (p[1]) estimated as those alleles that only occur in a single sampled meadow, observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F) according to Weir & Cockerham (1984). Tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed on individual meadows and the global dataset using GENEPOP v4.0.10 (Raymond & Rousset 1995).

Genetic structure among meadows

Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed using GENALEX 6.5 to determine the relative partitioning of total genetic variation into within and among sampled meadows. Differentiation among sampled meadows was estimated using F_{ST} following Weir & Cockerham (1984). Isolation by distance (IBD) across the sampled meadows was tested by a Mantel test of the correlation between genetic distance ($F_{ST}/1 - F_{ST}$) and geographic distance (km) in GENALEX 6.5. Geographic distance was calculated as the shortest distance between sampled meadows via water (derived oceanographic distance). A Principal Coordinates Analysis

Table 2. Summary of diversity indices for *Posidonia australis* meadows around Cockburn Sound. N = number of shoot samples; MLG = number of unique multilocus genotypes; MLL = number of multilocus lineages; *R* = clonal richness; *D** = Simpson diversity index; ED* = clonal evenness; β = slope of Pareto distribution (see Arnaud-Haond et al. 2007); N_a = number of alleles; *p*[I] = private alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; *F* = inbreeding coefficient; * = the 8 meadows included within Cockburn Sound; ** = significant deviation from Hardy-Weinberg equilibrium at p < 0.05

Pop.	Sample location	Abbr.	Clonal diversity (based on MLGs)							Genetic diversity (based on MLGs with missing data)					
			N	MLG	MLL	*R*	*D**	ED*	β	N	N_a	*p*[I]	H_o	H_e	*F*
1	Parker Point, Rottnest Is.	RPP	48	10	10	0.19	0.81	0.81	0.35	10	21	1	0.543	0.455	–0.214**
2	Fishing Boat Harbour	FB	48	38	36	0.79	0.98	0.70	1.17	39	46	2	0.511	0.514	0.009
3	Carnac Island	CI	50	43	16	0.86	0.99	0.84	2.49	43	27	1	0.306	0.300	–0.024
4*	Parmelia Bank 1 (west)	D1	44	16	15	0.35	0.94	0.96	0.88	16	32	2	0.545	0.529	–0.057**
5*	Parmelia Bank 2 (east)	D2	46	44	44	0.96	0.98	0.59	1.32	46	40	1	0.515	0.534	0.022
6*	Woodman Point 1	WP1	49	42	38	0.85	0.99	0.75	1.81	42	45	3	0.524	0.519	–0.018
7*	Woodman Point 2	WP2	44	37	37	0.84	0.98	0.58	1.33	43	40	0	0.478	0.504	0.068**
8*	Pig Trough Bay, Garden Is.	PT	50	39	34	0.78	0.98	0.78	1.03	39	28	0	0.469	0.433	–0.056**
9*	Walking Beach, Garden Is.	WB	48	25	24	0.51	0.96	0.92	1.12	25	35	0	0.520	0.482	–0.092**
10*	Southern Flats	SF	50	35	34	0.69	0.98	0.89	1.45	35	33	2	0.518	0.506	–0.034
11*	Mangles Bay	MB	49	20	18	0.40	0.90	0.79	0.49	20	27	0	0.529	0.470	–0.137**
12	Point Peron	PP	46	27	27	0.58	0.96	0.88	0.79	29	31	2	0.548	0.517	–0.037
13	Safety Bay	SB	49	20	19	0.40	0.94	0.90	0.75	20	30	1	0.514	0.535	0.053**
	Overall		621	393	–	0.63	–	–	–	407	73	–	0.502	0.485	–0.040**

(PCoA) was also performed in GENALEX 6.5 to visualize the relationship between individual MLGs and population means.

RESULTS

Within-meadow genetic diversity and structure

Complete MLGs for all 7 loci were obtained for 621 samples from 13 sampled *Posidonia australis* meadows, as some samples stored in silica gel did not genotype well and were excluded. There was no evidence for null alleles, stuttering, or large allele dropout in all but 3 meadows. Clonal diversity within meadows varied markedly (Table 2, Fig. 2). The number of MLGs ranged from 10 to 43 for between 44 and 50 samples. The number of MLGs and MLLs was very similar in all meadows, except Carnac Island (CI) where 38 pairs of MLGs differed by a single allele (Table 2). Heterogeneity among meadows was best reflected by MLGs (and MLLs) and β; clonal richness (*R*) was significantly associated with β (R^2 = 0.575, p = 0.002. Heterozygosity, *D**, and ED* did not appear to be as sensitive. The comparison of diversity estimates and sampling method shows sample locations largely fell into 2 main clusters — $R > 0.6$ and $R < 0.4$ — independent of sampling scheme (Fig. 2). Clonal richness measured at the Parmelia Bank D1 meadow was much lower than the adjacent Parmelia

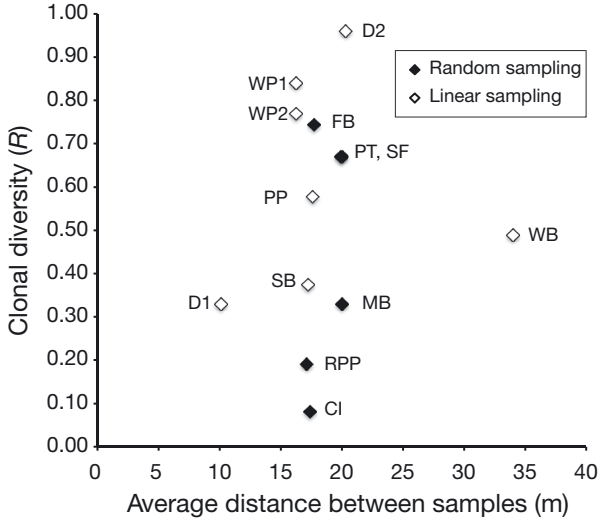

Fig. 2. Plot of average distance between samples against clonal diversity (*R*) (multilocus genotypes), where $R = (G - 1)/(N - 1)$ (*G* is the number of multilocus genotypes and N is the number of samples), showing low and high richness clusters and no association between randomly and non-randomly sampled meadows. Sample site codes are as in Fig. 1

Bank D2 meadow (R = 0.35 and 0.96, respectively), and likely a result of sampling at 1 m intervals. Similarly, the Walking Beach (WB) meadow was a low-diversity population after the effect of higher sampling interval on R was considered (Fig. 2).

Most meadows had a unique set of MLGs, with only 3 cases where a MLG was shared among meadows: (1) WP1 and WP2 = 0.4 km, (2) PT and MB = 16.1 km, and (3) PT and RPP = 20.6 km. The probability of obtaining the same MLG through a distinct sexual recombination event was very small (all p_{sex}< 0.01). Hence, identical MLGs within meadows were considered as pertaining to the same clone, while those from different meadows were regarded as different sexual events.

The 7 polymorphic loci had a total of 73 alleles with a range of 3 to 16 alleles per locus. While most meadows shared the most common alleles, 9 of the 13 meadows also had between 1 and 3 private alleles at low frequencies (<0.06). Overall, there was a significant association between the number of alleles and number of MLGs detected within each meadow (R^2 = 0.411, p = 0.018). However, when sites were partitioned according to sampling method, there was no association when a random sampling method was used and a highly significant association within linear sampled sites (R^2 = 0.736, p = 0.013). Overall, a moderate level of heterozygosity was observed (H_o = 0.50, Table 2). Levels of heterozygosity (H_o) were not significantly associated with the number of MLGs (R^2 = 0.226, p = 0.101). There were significant departures from HWE, with 8 of the 13 meadows out of HWE (Table 2). For LD, only 28 out of 261 tests were significant at p < 0.05 (10%), with no pattern across meadows or loci.

Genetic structure among meadows

There was significant genetic differentiation among the 13 sampled meadows, with an overall F_{ST} of 0.085 ± 0.009 (SE), and AMOVA partitioned 92% of the total genetic variation among individuals within meadows, and 8% among meadows (p < 0.001). Significant differentiation (F_{ST}) was also noted among most pairs of meadows (Table 3), with the exception of Woodman Point (WP1 and WP2) and Parmelia Bank (D1 and D2) meadows, which are all located at the northern end of Cockburn Sound and form part of an extensive meadow. Fishing Boat Harbour (FB) was also not significantly differentiated from WP1. A weak positive relationship exists between genetic distance ($F_{ST}/1 - F_{ST}$) and geographic distance (km) over all sampled meadows (n = 13; R^2 = 0.107, p = 0.085), a relationship that was considerably stronger among meadows within Cockburn Sound (n = 8; R^2 = 0.725, p < 0.001) (Fig. 3), although overall F_{ST} was weaker, but still significant (F_{ST} = 0.050 ± 0.008). A similar result was observed in the PCoA of sample means, in which those meadows that were significantly differentiated based on pairwise F_{ST} values were generally more distant than those that were undifferentiated (Fig. 4). The exceptions were CI, which has a high number of MLGs that are more similar to each other than MLGs from other sites (very tight clustering of CI MLGs relative to other sites in PCoA, not shown), and D1, which has significantly lower diversity as a result of sampling method (Fig. 2). The first axis accounts for 34% of the variation, in which the southernmost meadows are on the negative side of the first axis.

Table 3. Genetic differentiation (pairwise F_{ST}) between the 13 sampled *Posidonia australis* meadows. * = the 8 meadows included within Cockburn Sound; ** = p-values < 0.001. See Fig. 1 for site abbreviations

Pop.	Abbr.	RPP	FB	CI	D1	D2	WP1	WP2	PT	WB	SF	MB	PP	SB
1	RPP	–	**	**	**	**	**	**	**	**	**	**	**	**
2	FB	0.043	–	**	**	0.016	0.078	0.002	**	**	**	**	**	**
3	CI	0.128	0.116	–	**	**	**	**	**	**	**	**	**	**
4*	D1	0.049	0.025	0.064	–	0.132	0.001	0.002	**	**	**	**	**	**
5*	D2	0.051	0.010	0.090	0.008	–	0.398	0.623	**	**	**	**	**	**
6*	WP1	0.040	0.008	0.102	0.016	0.006	–	0.177	**	**	**	**	**	**
7*	WP2	0.056	0.013	0.093	0.015	0.005	0.007	–	**	**	**	**	**	**
8*	PT	0.049	0.034	0.091	0.035	0.034	0.034	0.035	–	**	**	**	**	**
9*	WB	0.051	0.028	0.074	0.021	0.020	0.025	0.018	0.020	–	**	**	**	**
10*	SF	0.082	0.050	0.123	0.039	0.035	0.044	0.039	0.047	0.035	–	**	**	**
11*	MB	0.118	0.079	0.111	0.052	0.055	0.070	0.060	0.061	0.041	0.024	–	**	**
12	PP	0.086	0.078	0.162	0.065	0.060	0.060	0.063	0.078	0.066	0.024	0.058	–	**
13	SB	0.047	0.025	0.126	0.036	0.028	0.027	0.032	0.027	0.037	0.036	0.067	0.059	–

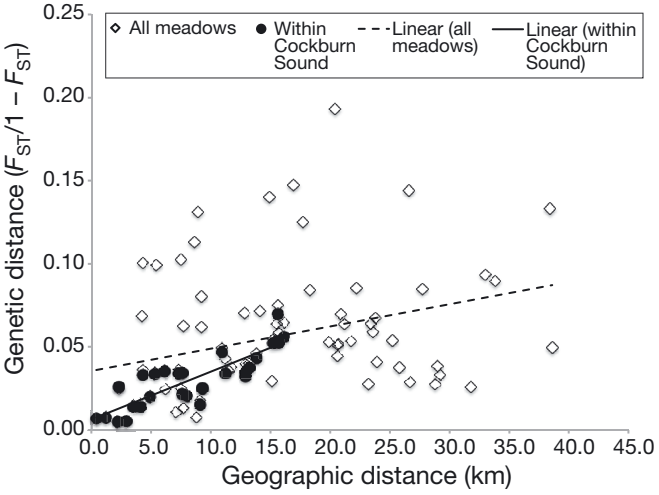

Fig. 3. Plot showing the relationship between genetic distance (as measured by F_{ST}/1 – F_{ST}) and geographic distance (km) over all pairs of sampled *Posidonia australis* meadows (Mantel test, R^2 = 0.107, p = 0.085; ◇), and among the 8 meadows sampled within Cockburn Sound (R^2 = 0.725, p < 0.001; ●)

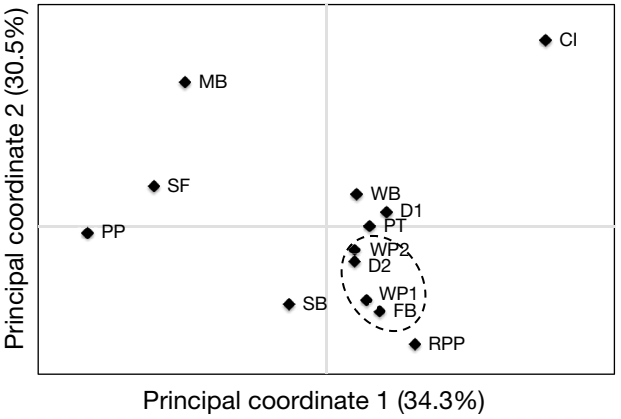

Fig. 4. Principal coordinates analysis (PCoA) showing the spatial separation by population means for the 13 *Posidonia australis* meadows. Sample site codes are as in Fig. 1. The dashed circle indicates those meadows that were not significantly differentiated based on pairwise F_{ST} values

DISCUSSION

What is driving local genetic diversity and structure within Cockburn Sound?

Our study focused on seagrass meadows largely within a semi-closed embayment, and highlights the difference between dispersal within inshore coastal shelf waters versus offshore oceanic currents, in which much less local structure is observed. We obtained significant differentiation (overall F_{ST} = 0.085) over 40 km of inshore coastal shelf habitat and within Cockburn Sound over ~16 km (F_{ST} = 0.050). The Cockburn Sound region is an area of high retention, sheltered from the direct influence of prevailing southwesterly winds. Water circulation rates within Cockburn Sound are extremely low, but wind forcing (stormy winds and sea breezes) creates surface currents (Steedman & Craig 1983). Particle tracking of these surface currents has provided insight into the dispersal of floating *Posidonia* seeds within Cockburn Sound (Ruiz-Montoya et al. 2012), which translates into gene flow among meadows, as well as the potential to restore damaged meadows through natural recruitment. Seeds are dispersed during the austral summer months when the prevailing winds from the southwest exert a direct force on the fruit (windage), with the majority of dispersing seeds transported northward via wind-driven surface currents (Ruiz-Montoya et al. 2012). This is consistent with the strong IBD relationship observed from genetic analyses within the Sound. This relationship breaks down once leaving the sheltered Sound—similar to that observed in the local sea urchin *Heliocidaris erythrogamma*, which has a short (planktonic) dispersal phase of 3 to 4 d, and no IBD relationship observed at distances over 10 km (Binks et al. 2011). A similar pattern was found in the seagrass *Zostera marina*, with significant IBD within a coastal lagoon (Muñiz-Salazar et al. 2006) and in the Gulf of California, but no IBD among more exposed meadows outside of the Gulf (Muñiz-Salazar et al. 2005).

Most sites showed strong genetic patchiness, possibly as a result of different seed-recruiting cohorts. This 'chaotic' genetic patchiness is a phenomenon increasingly reported to explain fine-grained genetic differentiation in a wide range of marine organisms, including seagrasses (Becheler et al. 2010, 2014) and intertidal species (Johnson & Black 1984, 2006, Johnson et al. 1993). Clonal diversity varies greatly among *Posidonia australis* meadows, although our estimates are considerably higher than those given in Waycott et al. (1997), and consistent with an emerging trend from microsatellite DNA data for seagrasses showing extreme variation among meadows (e.g. van Dijk et al. 2009, Serra et al. 2010). The β values reported here (0.35 to 2.49) indicate significant heterogeneity among meadows, with some meadows showing low-frequency widespread clones (e.g. RPP, MB, SB) and others with a high frequency of genets composed of a small number of ramets (e.g. CI, D2, WP2). The range in β values is also very similar to those exhibited in

P. oceanica meadows across the Mediterranean (Arnaud-Haond et al. 2010). This reflects a significant sexual contribution in a species that has typically been viewed as primarily clonal.

The capacity of a site to receive high numbers of (genetically diverse) seeds during the initial recruitment phase has a significant impact on genetic diversity within meadows. Floating fruit(s) form windrows across the ocean surface and move around by windage and surface water currents. Yet, we suspect that recruitment rates are very low over time (i.e. beyond the initial phase; Kendrick et al. 2012). Meadows at moderately sheltered to moderately exposed sites (based on Valesini et al. 2003; D1, D2, WP1, WP2, FB, PP) had higher levels of genetic diversity (R = 0.58 to 0.85), with the exception of D1. The highly sheltered sites (RPP, CI, WB, MB, SB) had generally lower levels of genetic diversity (R = 0.19 to 0.51), with the exception of CI, which had a high number of very closely related MLGs. Two possible exceptions are PT (R = 0.78), which is close to highly diverse meadows, and SF (R = 0.69), which is in a more exposed position at the southern opening to Cockburn Sound. The higher diversity meadows are in more exposed positions that are open to receiving a more diverse mix of seeds, while the lower diversity sites potentially receive less diverse material and/or potentially self-recruit (CI in particular). Both ISR and RSR strategies for recruitment will occur and depend strongly on local conditions and level of disturbance.

Other seagrass studies also suggest that local environmental conditions (disturbance, exposure) have a stronger influence on genetic diversity than meadow age or size (Procaccini et al. 1999, 2001, Rhode & Duffy 2004). Seagrass meadows can be extremely dynamic systems, with sediment constantly moving in more exposed sites, while other meadows may be extremely stable over long periods of time. A positive association between increasing clone size (and therefore reduced R) and lower hydrodynamic regimes was found in another seagrass, *Thalassia testudinum* (van Dijk & van Tussenbroek 2010). Genetic diversity will reflect a balance between enough exposure to regional current and wind patterns that would generate diversity through mixing of seed sources, but not too exposed such that seeds cannot recruit and establish. Patterns of genetic structure within and among meadows is thus the cumulative result of the impact of these local conditions, which ultimately modulate dispersal, successful seedling recruitment (addition of genetic diversity), and the extent of clonal growth.

Implications for restoration in semi-closed systems

Seagrass restoration is extremely difficult and time consuming, with many restoration projects being unsuccessful in the long term. Many of these projects have used vegetative material and given little or no attention to genetic diversity. Our study provides information on genetic diversity and gene flow in *P. australis* meadows within a relatively protected embayment, where seagrass has formed fairly continuous meadows, but seen dramatic declines in recent decades. Our genetic data suggests that gene flow occurs naturally throughout the Cockburn Sound region, but with IBD within Cockburn Sound. Significant differentiation among most meadows indicates the important role local environmental conditions play in the balance between sexual reproduction and recruitment and asexual growth. As seen in other seagrass species (e.g. Becheler et al. 2010, 2014), genetic patchiness is a balance between initial recruitment events and subsequent (low) rates of annual recruitment, vegetative growth, and the level of disturbance (Reusch 2006). It is the interaction of these events with local conditions that determine the extent to which natural recovery mechanisms impact on the persistence of meadows and the degree to which augmentation is required to initiate seagrass-meadow restoration following disturbance or removal. *Posidonia* spp. can be a primary colonizer and it may take decades for the seagrass community to recover to its former state (Bryars & Neverauskas 2004), although growth rates in *P. australis* appear more rapid on the west coast of Australia (Kendrick et al. 2008, Verduin et al. 2012) than the east coast (Meehan & West 2000). Some sites can recover naturally through both recruitment and vegetative growth (e.g. Kendrick et al. 2000), while others may not, particularly if significant changes to the seascape have occurred (e.g. Kendrick et al. 2002). These findings, along with inshore, localized coastal water circulation, are consistent with genetic patchiness observed among *P. australis* meadows and go a long way to explaining the local patchiness in distribution, clonal diversity, and genetic structure observed in this study. Restoration in the marine environment presents significant challenges for restoration ecologists, but this is where contributions from genetics in understanding patterns of spatial genetic structure and diversity are greatest.

Understanding local hydrodynamics, patterns of genetic diversity, seed dispersal distances, and causes of individual meadow decline will allow scientists and restoration ecologists to predict a meadows' abil-

ity to recover, and hence specific targeting of sites in most need of augmentation. Field methods are being developed for the use of *P. australis* seeds in restoration (Statton et al. 2013), which provides a large, although highly seasonal, genetically diverse resource for restoration. Recent and successful restoration of *Z. marina* meadows in the Chesapeake Bay area demonstrates the use of seeds (Orth et al. 2012) in a species with similar dispersal habits to *P. australis*. The use of locally available seeds excludes the need for screening for clonal diversity prior to sourcing vegetative transplants, as each seed is the result of a unique recombination event. Combining the use of vegetative material with seed material greatly reduces the reliance on personnel and damage to existing meadows. Traditional planting methods may be used strategically, as restoration of plants improves local conditions (e.g. McGlathery et al. 2012), so that subsequent seeding can enhance recruitment rates and meadow expansion. Genetic 'provenance' ranges could be set using the maximum seed dispersal distances (~55 km in 6 d; Ruiz-Montoya et al. 2012); thus genetic diversity at the regional scale becomes important at preserving gene flow and connectivity (Kinlan & Gaines 2003, van Dijk et al. 2009, Kendrick et al. 2012, Cornell & Harrison 2013). Our continuing research programme is shedding light on pollen and seed dispersal distances and developing a model to ensure that best practice in terrestrial plant restoration (e.g. Shackelford et al. 2013) will be transferred to successful long-term restoration of seagrass meadows.

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