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Genetic variation within and between populations of *Posidonia australis*, a hydrophilous, clonal seagrass

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Allozyme diversity was surveyed at 15 loci across 22 populations of the hydrophilous seagrass *Posidonia australis* (Hook. *f.*). Substantial genetic variation was detected ($H_T = 0.311$) with a high proportion of this variation partitioned between populations ($G_{ST} = 0.623$). The high value of G_{ST} is attributed to large geographical distances between many of the populations and several of the extreme north-western populations having fixed homozygous genotypes. South-western populations of *P. australis* were the most variable and these correlate with the highest species diversity in this genus. Intermediate levels of genetic diversity are observed in *P. australis* when compared with other hydrophilous angiosperms. Average gene diversity values for hydrophilous taxa surveyed to date indicate lower H_T and higher G_{ST} values than an average reported for 468 plant taxa. Patterns of genetic variability in different regions of the distribution of *P. australis* may reflect past evolutionary diversification into novel environments and subsequent dispersal following the rifting of Australia from Antarctica in the early Tertiary.

Keywords: allozymes, clonal, evolution, hydrophily, seagrass.

Introduction

Early population genetic studies on seagrasses and other hydrophilous angiosperms reported a lack of genetic variation and generalized that this was a feature of hydrophiles (McMillan, 1981, 1982, 1991; Wain *et al.*, 1985; Triest, 1991). Recently, a few thorough investigations of genetic variability in hydrophilous angiosperms have demonstrated significant diversity in both the level and distribution of genetic variation. Species such as *Ceratophyllum echinatum* are predominantly clonal with little variation (Les, 1991), whereas others such as *Zostera marina* have genetically variable populations comprising many individuals (Laushman, 1993). Several other studies on hydrophiles have investigated the fine-scale genetic structure of populations, documenting both multiclonal (Les, 1991; Laushman, 1993; Alberte *et al.*, 1994; Lokker *et al.*, 1994; Waycott, 1995) and apparently uniclonal (Les, 1991; Waycott *et al.*, 1996) populations. These results emphasize the need for thorough sampling to determine the level and extent of genetic variability within and between populations and to determine population demography and structure.

Posidonia australis (Hook. *f.*) is the broad-leaved form in a complex of three seagrass species (*P. australis*, *P. sinuosa* and *P. angustifolia*) previously described as '*australis*' (Cambridge & Kuo, 1979). *Posidonia australis* is widely distributed along the south-western, southern and south-eastern coasts of Australia (25°S,113°E to 31°S,135°E) with *P. sinuosa* and *P. angustifolia* being largely sympatric within its range. *Posidonia australis* is generally considered to be the climax species in sheltered nearshore sandy marine environments throughout its range and is a highly productive, long-lived perennial seagrass. Rhizome extension rates of *P. australis* are slow, of the order of 2.5 cm yr^{-1} (West, 1990) and seedling recruitment has not been observed (Kirkman, 1985; Kirkman & Kuo, 1990). As many regions of *P. australis* and other aquatic macrophytes are being dramatically affected by human impact through physical disturbance and elevated nutrient

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loads (Orth & Moore, 1983; Cambridge & McComb, 1984; Walker & McComb, 1992), increasing attention has been devoted to understanding the ecology and physiology of these organisms.

Knowledge of breeding systems and population genetic structure is necessary to understand the evolutionary processes shaping marine angiosperms and other hydrophiles (Barrett *et al.*, 1993; Waycott & Les, 1996). However, to date, only one published account of the genetic system of *P. australis* has been undertaken which identified significant within-population genetic variation within a small (400 m^2) meadow (Waycott, 1995). In this study we compare the allozymically determined genetic variability present in *P. australis* with other hydrophilous angiosperms and other plant species described in Hamrick & Godt (1989). The results are also discussed with reference to the process of meadow formation and the possible evolutionary pathways that have led to present-day species distributions in this genus.

Materials and methods

Collections and sample preparation

Collections were made from 22 populations of *P. australis* across its geographical range (Fig. 1). Samples were collected at distances greater than those described by Waycott (1995) in a study of genetic variation in a single population, to maximize the collection of different genets. Each collection had between nine and 70 ramets (on average 28 ramets per population) collected at least 5 m apart and within an area usually 200 × 200 m (but this was dependent on the extent of the meadow being sampled). After processing, samples were stored frozen at −80°C for up to 8 months until use.

Allozyme electrophoresis and data analysis

Horizontal starch gel electrophoresis was conducted according to Waycott (1995) using two buffer

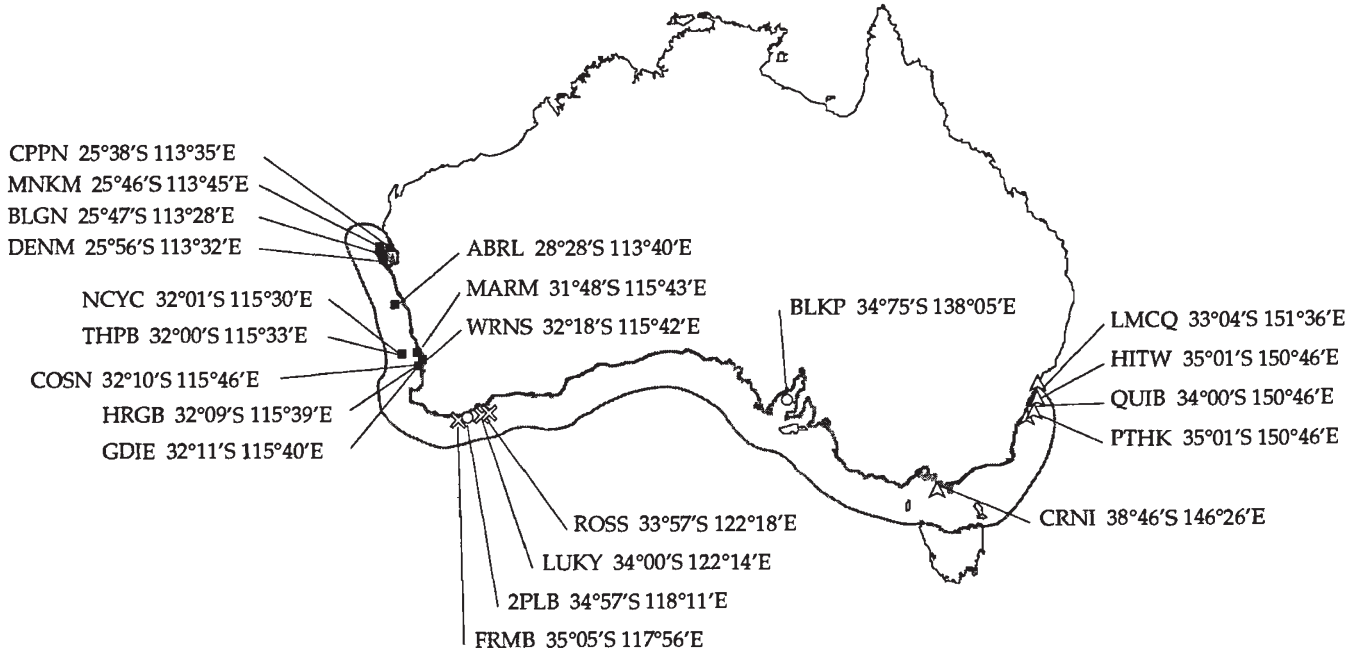

Fig. 1 Distribution of *Posidonia australis* in nearshore habitats over the range indicated by the outlined region. Population collections were made at the sites indicated with a symbol. Populations are indicated by their population code as follows in parentheses: Big Lagoon, Shark Bay (BLGN); Denham, Shark Bay (DENM); Cape Peron, Shark Bay (CPPN); Monkey Mia, Shark Bay (MNKM); Wallabi Island, Abrolhos (ABRL); Nancy Cove, Rottnest (NCYC); Thompsons Bay, Rottnest (THPB); Herring Bay, Garden Island (HRGB); Sulphur Bay, Garden Island (GDIE); Warnbro Sound, Rockingham (WRNS); Marmion Lagoon, Perth (MARM); Cockburn Sound, Rockingham (COSN); Frenchman Bay, Albany (FRMB); Two People Bay, Albany (2PLB); Lucky Bay, Esperence (LUKY); Rossiter Bay, Esperence (ROSS); Section Bank/Black Pole, Barker Inlet (BLKP); Little Snake Island, Corner Inlet (CRNI); 'Hole in the Wall', Jervis Bay (HITW); Costen's Point, Port Hacking (PTHK); Quibray Bay, Botany Bay (QUIB); Wangi Wangi Point, Lake Macquarie (LMCQ). Symbols on population locations refer to population clusters in Fig. 2.

systems, scoring a total of 15 loci from nine enzyme systems across all 22 populations (Table 1). Samples from the most polymorphic population, Lucky Bay, were run on all gels for comparative allelic designations.

Population genetic parameters were calculated for all samples as follows: allele frequencies, P (percentage polymorphic loci, 0.99 criterion), H_o (observed heterozygosity) and H_e (expected panmictic heterozygosity) were calculated using the computer program BIOSYS-1 (Swofford & Selander, 1981); A_e (effective number of alleles per locus) and P_s (total number of polymorphic loci at the species level) were calculated according to Hamrick & Godt (1989); M_l (number of multilocus genotypes) was calculated as the total number of different multilocus genotypes present in all the ramets sampled in each population. The multilocus genotype diversity statistic, D_G, which reflects the relative frequency of specific genotypes within the sampled pool of ramets, was calculated in the manner of Ellstrand & Roose (1987). A D_G value of one indicates all ramets have a unique multilocus genotype, whereas a value of zero indicates all ramets have a common multilocus genotype. The allelic diversity statistics of Nei (1978), H_S, H_T, D_{ST} and G_{ST} (for polymorphic loci) and UPGMA cluster analysis with standard errors were calculated using the computer programs, GD and GDD, of Ritland (1989), which calculate D_{ST} and G_{ST} from mean H_T and H_S values for species totals and individual polymorphic loci.

Results

A total of 634 samples was analysed from 22 populations; 15 loci were scored across all populations. Five loci were polymorphic, but the number of polymorphic loci per population varied from four in two populations (BLKP, MARM) to zero in six populations (GDIE, ABRL, BLGN, MNKM, CPPN, DENM) (Table 2).

The average number of detectable multilocus genotypes per population was 4.6, with a wide range from one genotype per population ($D_G = 0$) in eight populations to 19 multilocus genotypes identified in the LUKY population. In the LUKY population 73 per cent of the ramets had identifiably different genotypes. D_G values in three populations, ROSS, LUKY and MARM, were greater than 0.9, indicating many different genets without predominance of any one within the population (Table 3).

Allele frequencies of polymorphic loci are shown for all populations in Table 2. The maximum number of alleles per locus was four (*PGM-1*). The mean effective number of alleles per locus (A_e) was 1.07 and ranged from 1 to 1.26 (Table 3). The mean proportion of polymorphic loci per population (P) was 11 per cent, although the proportion of polymorphic loci for the species (P_s) was 33 per cent because some loci were polymorphic between populations. The average observed heterozygosity (H_o) was 0.045 and the expected panmictic heterozygosity (H_e) was 0.039. However, values ranged from zero in the genotypically uniform populations to $H_o = 0.131$

Table 1 Enzyme systems examined, the buffer systems used and the loci scored and observed (in parentheses). Starch buffer systems (MC, morpholine citrate; Moran & Hopper, 1983) run at 60 mA for 4.5 h; SAC/TC, Tris citrate modified (S. Carstairs, personal communication [100 mM Tris, 30 mM citric acid, 6.4 mM histidine (free base), 13 mM glycine, 5 mM Na_2EDTA, 2.5 mM $MgCl_2$, buffer to pH 7.4 with NaOH]) run at 65 mA for 5 h

Enzyme	Abbreviation	EC code	Buffer system	Loci scored (Loci observed)
α-Acid phosphatase	AAP	3.1.3.2	SAC/TC	1 (1)
Diaphorase	DIA	1.8.1.4	SAC/TC	— (2)
Fluorescent esterase	EST	3.1.1.-	SAC/TC	1 (1)
Glucose phosphate isomerase	GPI	5.3.1.9	SAC/TC	2 (2)
Isocitrate dehydrogenase	IDH	1.1.1.42	SAC/TC	1 (1)
Malate dehydrogenase	MDH	1.1.1.37	MC	4 (4)
Malic enzyme	MEN	1.1.1.40	MC	1 (1)
Menadione reductase	MR	1.6.99.-	SAC/TC	— (1)
Peroxidase	PER	1.11.1.7	MC	1 (3)
Phosphoglucomutase	PGM	5.4.2.2	SAC/TC	2 (3)
Phosphogluconate dehydrogenase	PGD	1.1.1.44	MC	1 (2)
Shikimic acid dehydrogenase	SDH	1.1.1.25	MC	1 (1)

Table 2 Allele frequencies at five polymorphic loci for 22 populations of *Posidonia australis*

Locus:	*IDH-1*			*AAP-1*			*PGM-1*				*PGD-2*			*SDH-1*	
Allele:	1	2	3	1	2	3	1	2	3	4	1	2	3	1	2
LMCQ	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.500	0.500	0.000	1.000	0.000	0.000	0.500	0.500
QUIB	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.862	0.138	0.000	1.000	0.000	0.000	0.017	0.983
PTHK	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	0.500	0.500
HITW	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	0.589	0.411
CRNI	0.000	0.000	1.000	0.000	0.350	0.650	0.000	1.000	0.000	0.000	1.000	0.000	0.000	0.714	0.286
BLKP	0.000	0.174	0.826	0.000	0.000	1.000	0.326	0.000	0.674	0.000	0.196	0.804	0.000	0.783	0.217
ROSS	0.276	0.000	0.724	0.000	0.000	1.000	0.207	0.190	0.569	0.034	0.690	0.069	0.241	0.000	1.000
LUKY	0.250	0.000	0.750	0.000	0.000	1.000	0.327	0.250	0.423	0.000	0.538	0.327	0.135	0.000	1.000
2PLB	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.016	0.984	0.000	0.710	0.290	0.000	1.000	0.000
FRMB	0.000	0.000	1.000	0.000	0.308	0.692	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000
WRNS	0.000	0.000	1.000	0.000	0.940	0.060	0.080	0.000	0.920	0.000	1.000	0.000	0.000	0.020	0.980
HRGB	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.056	0.944	0.000	1.000	0.000	0.000	0.167	0.833
GDIE	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000
COSN	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.389	0.611	0.000	1.000	0.000	0.000	0.153	0.847
THPB	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.467	0.533	0.000	0.867	0.033	0.100	0.150	0.850
NCYC	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.431	0.569	0.000	1.000	0.000	0.000	0.194	0.806
MARM	0.000	0.000	1.000	0.027	0.973	0.000	0.000	0.486	0.514	0.000	0.932	0.000	0.068	0.284	0.716
ABRL	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000
BLGN	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000
MNKM	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000
CPPN	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000
DENM	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000

and $H_e = 0.110$ in LUKY, the most genotypically diverse population (Table 3). The average values were different from those calculated for 468 species presented by Hamrick & Godt (1989), of $A_e = 1.15$ and $H_e = 0.113$. Fixation was observed at some loci in some populations (Table 4) but significant deviations of F from zero occurred in only five out of a possible 36 cases (14 per cent). These loci were either fixed as observed in the two polymorphic loci *PGM-1* and *SDH-1* in LMCQ or represented a complete lack of heterozygotes within polymorphic populations such as *AAP-1* in FRMB. Overall there was a mix of positive and negative F-values (Table 4). The six populations which were completely monomorphic all had the same multilocus genotype (Table 2).

Genetic diversity statistics calculated for individual polymorphic loci and as a mean for all polymorphic loci (Table 5) demonstrate a high G_{ST}, indicating that, on average, 62.3 per cent of the diversity was between populations. The high G_{ST} value for the *AAP-1* locus (0.902) is attributable to the fixation of alleles *2* and *3* in different populations (see Table 2). The mean G_{ST} for all polymorphic loci is 0.494 if *AAP-1* is excluded. The mean H_T is 0.311, ranging from 0.063 at *IDH-1* to 0.491 at *AAP-1*.

The maximum genetic distance between populations was 0.116 and UPGMA analysis demonstrated a general geographical trend from the eastern to southern to western populations (Fig. 2). Three significant clusters of populations occur. The western populations form one group and two different clusters of eastern and southern populations another group (Fig. 2). The trend generally follows a geographical gradient with more closely located populations clustering with each other rather than with those farther away. The western group of populations reflects the high level of genetic similarity, particularly the occurrence of identical homozygous allozyme genotypes in the Shark Bay populations (DENM, CPPN, MNKM, BLGN), the island population from the Abrolhos Islands (ABRL) and one Garden Island population (GDIE).

Table 3 Genetic diversity within populations of *Posidonia australis*

Population	n	P	A_e	H_o	H_e	M_l	D_G
LMCQ	30	13.3	1.13	0.133 (0.091)	0.068 (0.046)	1	0.000
QUIB	29	13.3	1.02	0.021 (0.018)	0.018 (0.016)	3	0.431
PTHK	29	6.7	1.07	0.067 (0.067)	0.034 (0.034)	1	0.000
HITW	28	6.7	1.06	0.055 (0.055)	0.033 (0.033)	2	0.304
CRNI	70	13.3	1.10	0.062 (0.044)	0.058 (0.040)	8	0.815
BLKP	23	26.7	1.14	0.099 (0.054)	0.094 (0.043)	4	0.688
ROSS	29	20.0	1.19	0.110 (0.060)	0.099 (0.054)	13	0.924
LUKY	26	20.0	1.26	0.131 (0.073)	0.110 (0.060)	19	0.969
2PLB	31	13.3	1.05	0.037 (0.034)	0.030 (0.028)	4	0.578
FRMB	26	6.7	1.05	0.000 (0.000)	0.029 (0.029)	2	0.443
WRNS	25	20.0	1.02	0.021 (0.013)	0.020 (0.012)	5	0.423
HRGB	9	13.3	1.03	0.030 (0.023)	0.027 (0.020)	3	0.556
GDIE	9	0.0	1.00	0.000 (0.000)	0.000 (0.000)	1	0.000
COSN	36	13.3	1.08	0.050 (0.035)	0.050 (0.035)	4	0.729
THPB	30	20.0	1.11	0.040 (0.024)	0.067 (0.039)	7	0.789
NCYC	35	13.3	1.09	0.035 (0.027)	0.054 (0.038)	6	0.790
MARM	37	26.7	1.13	0.061 (0.038)	0.073 (0.042)	13	0.910
ABRL	35	0.0	1.00	0.000 (0.000)	0.000 (0.000)	1	0.000
BLGN	12	0.0	1.00	0.000 (0.000)	0.000 (0.000)	1	0.000
MNKM	35	0.0	1.00	0.000 (0.000)	0.000 (0.000)	1	0.000
CPPN	9	0.0	1.00	0.000 (0.000)	0.000 (0.000)	1	0.000
DENM	40	0.0	1.00	0.000 (0.000)	0.000 (0.000)	1	0.000
Average	28	11 ($P_s = 33$)	1.07	0.045	0.039	4.6	0.425

P, percentage polymorphic loci (frequency <0.99); n, number of ramets sampled; A_e, effective number of alleles per locus; H_o, observed heterozygosity; H_e, expected panmictic heterozygosity; M_l, number of multilocus genotypes; D_G, multilocus genotype diversity; P_S, percentage polymorophic loci across all populations; standard errors in parentheses.

Table 4 Fixation indices (F) for polymorphic loci in each *Posidonia australis* population surveyed

	Locus				
Population	*PGM-1*	*SDH-1*	*AAP-1*	*IDH-1*	*PGD-2*
LMCQ	−1.000*	−1.000*	—	—	—
QUIB	−0.160	−0.018	—	—	—
PTHK	—	−1.000*	—	—	—
HITW	—	−0.697	—	—	—
CRNI	—	−0.160	−0.287	—	—
BLKP	−0.484	−0.278	—	1.000*	−0.243
ROSS	−0.157	—	—	−0.208	−0.046
LUKY	−0.121	—	—	−0.128	−0.381
2PLB	−0.016	—	—	—	−0.253
FRMB	—	—	1.000*	—	—
WRNS	−0.087	−0.020	−0.064	—	—
HRGB	−0.059	−0.200	—	—	—
GDIE	—	—	—	—	—
COSN	0.065	−0.180	—	—	—
THPB	0.866	−0.176	—	—	0.019
NCYC	0.717	−0.241	—	—	—
MARM	0.459	−0.263	—	—	−0.072
ABRL	—	—	—	—	—
BLGN	—	—	—	—	—
MNKM	—	—	—	—	—
CPPN	—	—	—	—	—
DENM	—	—	—	—	—

*Significant departures from zero for chi-squared tests of F-values using Šidák's correction ($P < 0.0004$) (Šidák, 1967).

These populations, plus the other Garden Island population (HRGB) and the Warnbro Sound population (WRNS), share a single most common genotype which clusters them tightly. There is a considerable increase in the number of polymorphic loci and the number of alleles present in the southern and eastern populations which contributes to the higher genetic distances observed between these populations. The populations at the extremes of the range of *P. australis* tend to be more homozygous and genetically uniform.

Discussion

The proportion of genetic variation between populations ($G_{ST} = 0.623$) in *P. australis* compared with the mean for 468 taxa (Hamrick & Godt, 1989) is high (Table 6). Among the hydrophiles *P. australis* has intermediate levels of genetic diversity (Table 6).

Table 5 Genetic diversity statistics for polymorphic loci of *Posidonia australis* and total diversity statistics for the species

Locus	H_T	H_S	D_{ST}	G_{ST}
IDH-1	0.063	0.050	0.013	0.206
AAP-1	0.491	0.048	0.443	0.902
PGM-1	0.481	0.217	0.264	0.549
PGD-2	0.174	0.099	0.075	0.431
SDH-1	0.354	0.176	0.178	0.502
Total for species	0.311	0.117	0.194	0.623

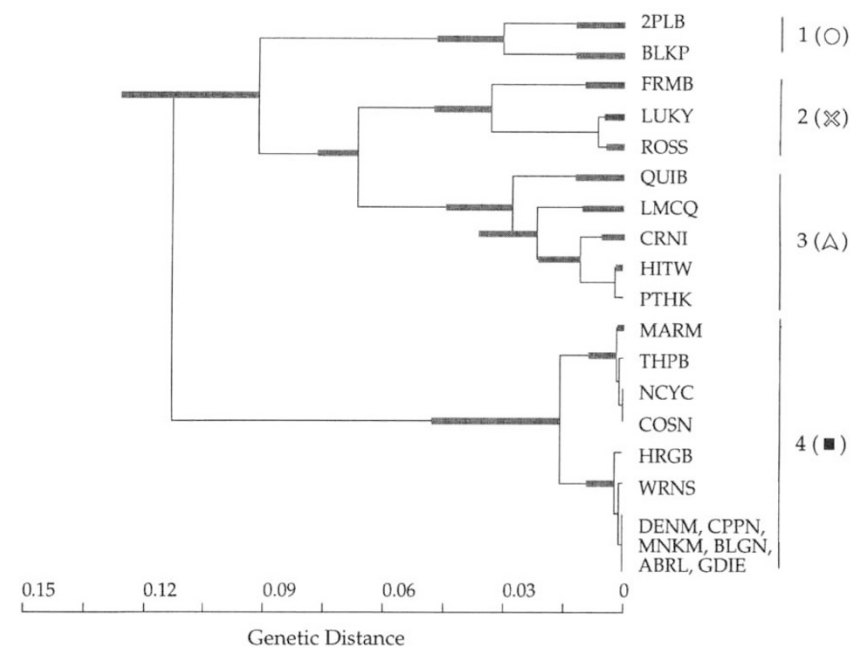

Fig. 2 UPGMA analysis of genetic distance (Nei, 1978) with standard errors (shaded bars), where a significant cluster occurs if the shaded bar is less than half the branch length (Ritland, 1989). Populations in the top cluster form a group from eastern and southern populations, whereas the lower cluster forms a group from western populations (see Fig. 1 for locality details). Symbols for the major statistically significant clusters are provided for reference to localities in Fig. 1.

Values for P and H_e are significantly lower in *P. australis*, and are near the average for H_T, compared with the categories proposed by Hamrick & Godt (1989). Low values for H_e and high G_{ST} in *P. australis* together distinguish this species from most of the proposed categories. The only category with a similar G_{ST} value is that of a selfing breeding system, although selfing species are usually associated with a higher H_e (0.149) than observed in *P. australis* (0.039). If the summary results are used as predictors of breeding system, then they suggest that *P. australis* has a selfing breeding system. However, although this species has the potential to self-pollinate, being functionally hermaphrodite, concurrent studies have identified high outcrossing rates in several populations (Waycott & Sampson, 1997).

Equivalent allozyme diversity was not found in eastern and western populations of this species. Some populations, particularly from the north-western extremes of the range (the Shark Bay populations), have a common, homozygous, multilocus genotype. South-western Australian populations of *P. australis* exhibit considerably more genetic variability, having higher levels of allelic variation, greater number of polymorphic loci, and high D_G values indicating a greater number of genets per population. South-eastern populations are variable in some cases but intermediate to the two westerly regions. UPGMA analysis revealed a trend which is driven by the fixed genotype observed in the homozygous north-western populations and the very widely spaced population collections, whereby large differentiation between populations may be expected.

The occurrence of many populations with a common homozygous multilocus genotype (DENM, CPPN, MNKM, BLGN, ABRL, GDIE) may be interpreted in two ways. First, it may represent the result of a limited founding population (Barrett & Shore, 1989) from tight genetic bottlenecks during the process of range extension. On the west coast prevailing currents are from north to south and the long-distance movements of short-lived propagules (mature fruit, see Kuo, 1982) are unlikely. This makes dispersal to new, more northerly environments improbable. Thus, a genotypically limited founding population might give rise to a widespread homozygous genotype through inbreeding. The second hypothesis is that these populations are highly clonal. Should this be the case, clones may be very large and extremely old. This hypothesis had been proposed earlier because of the infrequent or zero recruitment and slow recovery of populations following disturbance (Kirkman & Kuo, 1990). However, it has already been demonstrated that allozymes underestimate the level of variation present in this species (Waycott, 1995) and homozygous populations need not be entirely vegetative. Also, sexual reproduction is common in many of these populations (Walker, 1989), even if recruitment from sexual propagules is rare. In all, the lack of variability is more likely to be caused by founder effects and inbreeding.

Significant variation in the extent and distribution of genetic variation has been demonstrated in studies on a number of terrestrial clonal plant species and may be expected among species with a variety of sexual and asexual reproductive strategies

Table 6 Comparison of genetic diversity with other hydrophilous angiosperms (based on polymorphic loci for species)

Species	No. of Populations	No. of Loci	P	H_e	H_T	G_{ST}
Posidonia australis[1]	22	15	33.3	0.039	0.311	0.623
Ceratophyllum demersum[2]	9	17	20.0	0.064	0.211	0.495
Ceratophyllum echinatum[2]	3	17	7.0	0.036	0.529	0.481
Zostera marina[3]	7	24	38.5	0.085	—	0.457
Amphibolis antarctica[4]	7	14	0.0	0.000	0.000	—
Average for true hydrophiles	10.25	18.25	19.76	0.045	0.26*	0.514†
Summary of 468 taxa[5]	12.70	16.50	34.20	0.113	0.31	0.224

[1] This study, [2]Les(1991), [3]Laushman (1993), [4]Waycott *et al.* (1996), [5]Hamrick & Godt (1989). *Averaged for values reported only.
†Not calculated for *A. antarctica* because G_{ST} cannot be calculated for this species as no differentiation was observed.

(Ellstrand & Roose, 1987; Murawski & Hamrick, 1990; Les, 1991; Eckert & Barrett, 1993). The relative levels of sexual and asexual reproduction can be affected by both ecological and genetic factors (Ellstrand & Roose, 1987; Eckert & Barrett, 1993) and may be expected to determine the patterns of genetic diversity in population systems. Many monocotyledons have a propensity for clonal growth through rhizome extension. In particular, perennial aquatic plants have well-developed vegetative reproduction and often only rare sexual recruitment (Les, 1988) and may be expected to exhibit reduced levels of genetic diversity. However, hydrophiles appear to have reasonably high levels of diversity compared to flowering plant species generally, although they commonly have lower P, H_e and H_T but higher G_{ST} values. There are only a few studies directly comparable to this one among hydrophiles, with two on seagrass (*Zostera marina*, Laushman, 1993; *Amphibolis antarctica*, Waycott *et al.*, 1996). The results presented here are for a widely distributed, hermaphrodite seagrass and show that *P. australis* is genetically diverse (allozyme analysis) like the monoecious *Z. marina*, whereas the dioecious *A. antarctica* is genetically uniform for allozyme and M13DNA fingerprint analysis. These observations suggest that the breeding system does not solely determine the extent of genetic diversity within seagrass species as has been previously suggested (Pettitt *et al.*, 1981; Cox, 1993).

The highest level of genetic diversity in *P. australis* occurs within the region of greatest species richness where all eight described Australian species are known to coexist (Kirkman & Walker, 1989). The environments along the southern coast of Australia, particularly in the western regions, are extremely heterogeneous and possess some of Australia's harshest coastline. This region is approximately that which joined the Australian land mass to Antarctica in Gondwanan times and the rift valley which formed is immediately off the Western Australian southern coast (Quilty, 1994). Prior to the rift valley forming, the main refugia for marine organisms would either have been in what are now more tropical waters in north-western Australia, or in eastern waters and south of Tasmania in what is now part of Antarctica. For *Posidonia* to move from either source into what is its present distribution would have required it to evolve mechanisms to cope with a wide range of environments from near-estuarine to open ocean conditions.

The absence of comparable selective pressures in the Mediterranean, where the other extant *Posidonia* occurs (*P. oceanica*), may help to explain the paucity of *Posidonia* species in the northern hemisphere. The hydrodynamic environment off the southern Australian coastline is characterized by strong currents and high wave energy which may have promoted strong selection pressures leading to the divergence of the *P. ostenfeldii* 'complex' and the *P. australis* 'complex' (see Cambridge & Kuo, 1979 and Kuo & Cambridge, 1984 for details on the two Australian *Posidonia* species complexes). In this scenario, *P. australis* might be one of a relatively rapidly evolving group in the process of increasing its range. This may explain the homogeneity observed in the north-western distribution extremes of this species (assuming range extension from the south) as discussed above. An alternative hypothesis for the genetic diversity in southern populations is that relatively recent hybridization has occurred between species in southern populations. It should be noted, however, that these species occur together for much of the western distribution and equivalent hybridization and diversity would be expected in these environments if this hypothesis were true. However, hybridization between east and west components of a widespread ancestral seagrass following separation of Antarctica and the Australian continent is another hypothesis which could explain both the genetic diversity within *P. australis* and the taxonomic diversity within the Australian representatives of this genus.

The *P. australis* 'complex' of three species shares many characteristics with the Mediterranean species *P. oceanica*, including morphology and ecological niches, and is less similar to the other Australian group, the *P. ostenfeldii* 'complex'. It is possible that the *P. australis* 'complex' and *P. oceanica* may be more similar to an ancestral type than either are to the *P. ostenfeldii* 'complex'. The species investigated in this study, *P. australis*, has the widest distribution of all the Australian *Posidonia* species. The differences in distribution of the Australian members of this genus may simply reflect the availability of suitable environments for each species. However, the extension of *P. australis* farther east than any other species may indicate its progenitor status. The evolution of these *Posidonia* species complexes is of considerable interest given the paucity of species diversity among the seagrasses as a whole, their ancient fossil record and apparent conservatism, with extant species identified in Eocene fossils (Larkum & den Hartog, 1989). The observation presented here of elevated levels of genetic variation in *P. australis* coinciding with the region of greatest sympatry in the genus may well be meaningful, and warrants further investigation.

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