MOLECULAR ECOLOGY

Molecular Ecology (2012) doi: 10.1111/j.1365-294X.2012.05500.x

Range-edge genetic diversity: locally poor extant southern patches maintain a regionally diverse hotspot in the seagrass *Zostera marina*

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Abstract

Refugial populations at the rear edge are predicted to contain higher genetic diversity than those resulting from expansion, such as in post-glacial recolonizations. However, peripheral populations are also predicted to have decreased diversity compared to the centre of a species' distribution. We aim to test these predictions by comparing genetic diversity in populations at the limits of distribution of the seagrass *Zostera marina*, with populations in the species' previously described central diversity 'hotspot'. *Zostera marina* populations show decreased allelic richness, heterozygosity and genotypic richness in both the 'rear' edge and the 'leading' edge compared to the diversity 'hotspot' in the North Sea/Baltic region. However, when populations are pooled, genetic diversity at the southern range is as high as in the North Sea/Baltic region while the 'leading edge' remains low in genetic diversity. The decreased genetic diversity in these southern Iberian populations compared to more central populations is possibly the effect of drift because of small effective population size, as a result of reduced habitat, low sexual reproduction and low gene flow. However, when considering the whole southern edge of distribution rather than per population, diversity is as high as in the central 'hotspot' in the North Sea/Baltic region. We conclude that diversity patterns assessed per population can mask the real regional richness that is typical of rear edge populations, which have played a key role in the species biogeographical history and as marginal diversity hotspots have very high conservation value.

Keywords: diversity hotspots, edge populations, seagrasses, *Zostera marina*

Received 3 November 2011; revision received 15 January 2012; accepted 17 January 2012

Introduction

Present species distributions in Europe have been greatly influenced by climate changes resulting from advances and retreats of ice sheets during the last glacial maximum, 18 000 years ago (e.g. Van den Hoek *et al.* 1990; Hewitt 1993, 1996). These and the corresponding changes in sea level have also left their signature on the distribution of marine species, causing range expansion of taxa from southern Atlantic refugia into newly opened areas (reviewed by Maggs *et al.* 2008).

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Several glacial refugial areas have been identified in the south of Europe through combined analysis of palaeoecological and genetic data, and for some species, the recolonization route after the LGM has been reconstructed (Taberlet *et al.* 1998; Hewitt 2004). Theory predicts that southern refugial areas will harbour the highest genetic diversity with attenuation northwards as a consequence of founder events, which represent subsets of the larger diversity of the refugial source population (Hewitt 1996; Ibrahim *et al.* 1996; Widmer & Lexer 2001). Range expansion of populations ('leading edge') to the north will result in low diversity dominated by fewer genotypes and a high frequency of alleles identical to or descended from the founding population (Hewitt 1996, 1999, 2004; Ibrahim *et al.* 1996;

Bernatchez & Wilson 1998). Leading edge populations are also predicted to be less structured than refugial populations. This has been found repeatedly in European terrestrial species (Hewitt 2000, 2001), and evidence is accumulating for marine species (reviewed in Maggs *et al.* 2008; see also Neiva *et al.* 2010; Coyer *et al.* 2011, Provan & Maggs 2012).

Diversity patterns in edge populations are also shaped by other post-glacial events such as population bottlenecks, secondary contact zones and cryptic refugia (Brochmann *et al.* 2003; Petit *et al.* 2003; Provan & Bennett 2008). Former refugial areas may now find themselves in the periphery of their distribution (e.g. Neiva *et al.* 2012). These 'rear edge' (trailing edge) populations are typically restricted to particular habitat islands within a matrix of unsuitable landscapes (Hampe & Petit 2005). In range-edge populations, the scarcity of suitable habitat may be reflected in small population sizes and lower gene flow, which may result in increased selfing and inbreeding and an increase in the incidence of clonal reproduction (e.g. Billingham *et al.* 2003; Arnaud-Haond *et al.* 2006; Beatty *et al.* 2008). These factors may decrease genetic diversity and may cause loss of adaptive potential (e.g. Pearson *et al.* 2009), a concern which is particularly relevant today with the current global climate change and its effect on species' distributional ranges (Thomas *et al.* 2004). However, disproportionately high levels of genetic differentiation observed among such populations may actually lead to exceptionally high levels of regional genetic diversity (Comps *et al.* 2001; Castric & Bernatchez 2003; Hampe *et al.* 2003; Petit *et al.* 2003; Martin & McKay 2004; Neiva *et al.* 2010).

In the present study, we aim at investigating which of the above scenarios holds for the rear edge populations of the temperate Atlantic seagrass species *Zostera marina* by analysing genetic structure and diversity at the edges of the distribution and compare results with earlier studies from the North Sea/Baltic range of the distribution, which is considered a diversity 'hotspot' (Olsen *et al.* 2004) and with populations at the 'leading edge' (northern latitudinal limit).

Zostera marina is the most widely distributed marine plant in temperate regions of both Pacific and Atlantic coasts in the Northern Hemisphere. In Europe, *Z. marina* is the only seagrass to extend into the Arctic Circle, and its southern latitudinal limit occurs on the south coast of the Iberian Peninsula. Like other seagrasses, *Z. marina* reproduces sexually through hydrophilous pollination and seeds and vegetatively by horizontal rhizomes bearing new leaf shoots. The 'geographical parthenogenesis' hypothesis predicts that vegetative (clonal) reproduction becomes predominant at distributional edges (e.g. Eckert 2002). There is growing evidence that indeed sexual reproduction is strongly reduced in rear edge populations of *Z. marina* (Billingham *et al.* 2003; Cabaço & Santos 2010).

Genotypic richness, an indicator of the relative importance of sexual vs. clonal growth for population dynamics, is expected to correlate, across geographical distributional ranges, with climate oscillations and central–peripheral distribution patterns. Yet, we still understand only poorly the distribution patterns of genotypic richness for marine clonal organisms with mixed sexual and asexual reproductive modes (but cf. Billingham *et al.* 2003; Coyer *et al.* 2004; Olsen *et al.* 2004; Tatarenkov *et al.* 2005; Alberto *et al.* 2006).

Populations of *Z. marina* from the North Sea/Baltic region were found to exhibit higher genetic richness than the populations at the limits of the species distribution (Olsen *et al.* 2004). This was proposed to reflect a possible secondary contact zone, mixing Eastern Atlantic with Western Atlantic *Z. marina*. However, there is a large difference in sampling effort in terms of number of sites, when comparing this North Sea/Baltic diversity 'hotspot' with peripheral populations, creating the possibility of an underestimation of the diversity at the periphery of the distribution, if most of the diversity is between populations rather than within.

In this study, we use a more extended sampling at the southern edge to re-test the hypothesis that the fragmented populations of *Z. marina* at the southern limit of distribution show decreased genetic diversity and increased differentiation compared to the northern part of the distribution. This is predicted owing to high genetic drift and probable low effective population size as a result of shifting from sexual to asexual (clonal) reproduction. However, we hypothesize that the high diversity expected in refugial populations is still present and that when the 'rear edge' is considered globally integrating many single populations, its diversity equals or exceeds that of the North Sea/Baltic region. We test these hypotheses by analysing genetic diversity and differentiation in all existing *Z. marina* populations at the distributional margin (southern Iberia), and we then compare the results with published data from populations at the diversity hotspot in the North Sea/Baltic range of the distribution (Olsen *et al.* 2004) and with populations from the leading edge.

Material and methods

Sample collection

Two data sets were used in this study (Table 1 and Fig. 1). The first data set consists of samples from two climatic extremes: four populations from Greenland, the 'leading' edge, and 10 populations from the 'rear' edge

Table 1 Names of populations sampled in each geographical region, their coordinates and population genetic parameter estimates

	Sampling site	Country	Abbreviation	Latitude	Longitude	*N*	*G*	*R*	Alleles/locus	Â (G = 10)	SE	H_E	H_O	F_{is}	Significance
Leading edge															
1	Kappisilit	Greenland	Kapi	64°28.096′N	50°13.746′W	30	8	0.24	2.25	NA	NA	0.3223	0.4531	**−0.349**	***
2	Amerillik 3	Greenland	Amer3	64°20.792′N	50°25.336′W	30	25	0.83	3.00	2.39	0.21	0.2484	0.245	0.034	
3	Amerillik 1	Greenland	Amer1	64°15.359′N	50°35.015′W	30	17	0.55	2.25	2.03	0.10	0.2011	0.1912	0.079	
4	Kobbefjord	Greenland	Kobbe	64°09.680′N	51°33.377′W	29	1	0	1.38	NA	NA	0.1875	0.375	**0**	***
					Region pooled	119	51	0.42							
Diversity hotspot															
5	Falkenstein	Germany	Falk	54°24′N	10°12′E	80	36	0.44	6.88	4.59	0.28	0.5262	0.5063	0.052	
6	Maasholm	Germany	Maas	54°41′N	10°00′E	110	46	0.41	7.13	4.73	0.36	0.4739	0.4281	**0.107**	***
7	Munkmarsch	Germany	SyltM	54°53.979′N	8°22.139′E	48	48	1	9.63	5.73	0.31	0.6658	0.613	**0.089**	***
8	Langeness	Germany	LangrN	54°39′N	08°32′E	35	35	1	6.00	4.67	0.30	0.6275	0.5591	**0.123**	***
9	Hooge	Germany	HoogeT	54°32′N	08°01′E	48	47	0.98	8.75	5.69	0.46	0.6331	0.613	0.042	
10	Groningen	Netherlands	Gron2	53°22.000′N	6°56.000′E	50	50	1	7.63	4.71	0.29	0.5351	0.4548	**0.159**	***
					Region pooled	371	262	0.71							
Rear edge															
11	Ponta do Adoche	Portugal	PA	38°29.525′N	08°54.507′W	86	76	0.88	6.50	3.92	0.43	0.5186	0.4414	**0.14**	***
12	Tróia	Portugal	Troia	38°28.911′N	08°54.337′W	317	92	0.29	6.63	4.22	0.32	0.5515	0.5122	**0.073**	***
13	Arrábida	Portugal	Arr	38°28.613′N	08°58.796′W	33	4	0.09	2.50	NA	NA	0.3906	0.5313	−0.286	
14	Sado	Portugal	Sado	38°27.829′N	08°51.617′W	28	6	0.19	1.88	NA	NA	0.3091	0.55	**−0.78**	***
15	VN de Milfontes	Portugal	VNM	37°43.324′N	08°46.612′W	125	64	0.51	5.63	3.84	0.34	0.4728	0.4736	−0.008	
16	Fuzeta	Portugal	Fuzeta	37°3.277′N	07°43.716′W	43	12	0.26	2.63	2.56	0.06	0.3594	0.3958	0.002	
17	Esteiro do Baião	Portugal	EstB	37°01.017′N	07°59.790′W	40	26	0.64	4.25	3.33	0.22	0.4216	0.4663	−0.086	
18	Culatra	Portugal	PLS	37°0.080′N	07°49.300′W	48	42	0.87	4.38	3.04	0.32	0.3673	0.3942	−0.062	
19	Barrinha	Portugal	Bar	36°58.760′N	07°56.757′W	65	23	0.34	3.75	3.22	0.22	0.3815	0.4923	**−0.279**	***
20	Cadiz	Spain	Cadiz	36°28.057′N	06°14.921′W	40	31	0.77	5.00	4.02	0.33	0.4975	0.4556	**0.158**	***
					Region pooled	825	376	0.46							

Number of ramets (*N*), number of genets (*G*), genotypic diversity (*R*), standardized allelic richness (*G* = 10) and standard error (SE), expected and observed heterozygosity (H_E and H_O) and the inbreeding coefficient (F_{IS}) with significance levels (***$P < 0.001$).

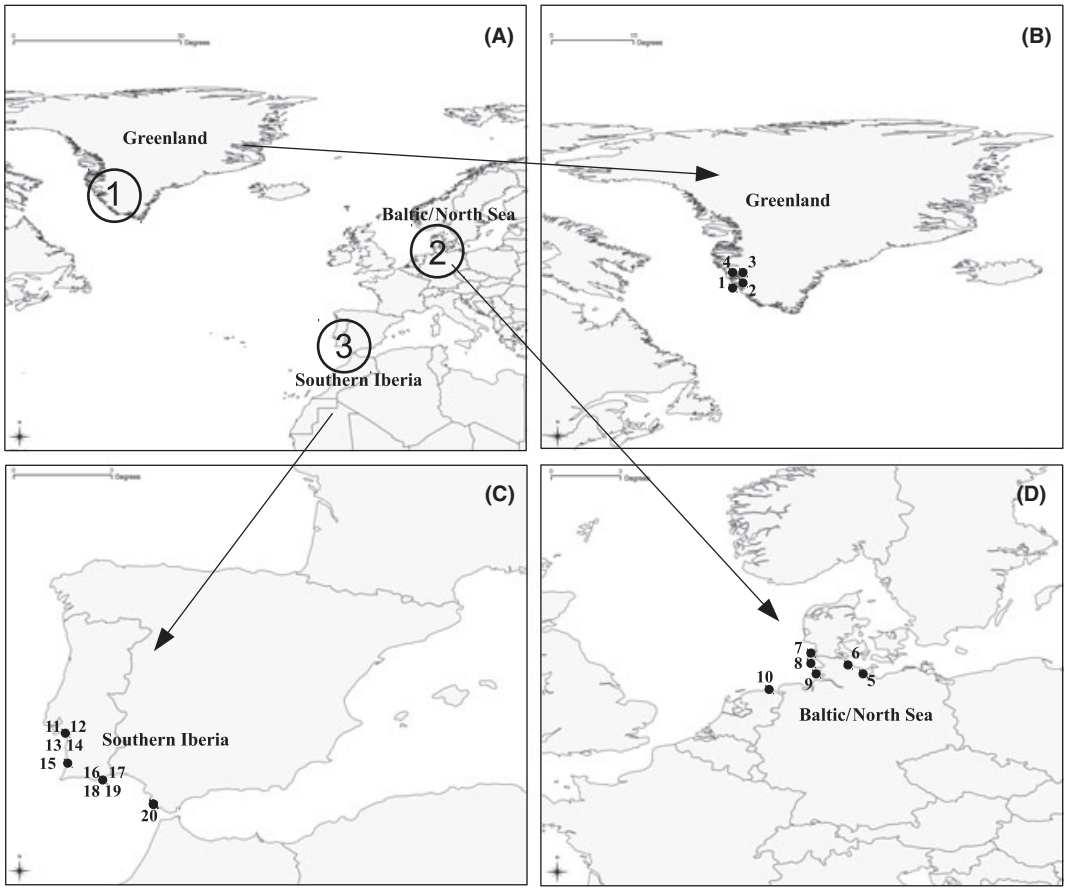

Fig. 1 Map showing regions and sampling locations. Map A is an overview of the North Atlantic, (1) represents the 'leading' edge, (2) is the North Sea/Baltic diversity 'hotspot' and (3) is the southern limit of distribution or 'rear' edge. Maps B, C and D show sampling locations within each region (for details of the populations indicated by the numbers, see Table 1).

at the southern limit of distribution in the Iberian Peninsula. Although the number of populations is different between edges, it adequately represents all the diversity that exists in each region because all populations were sampled within each region; at their northernmost limit in Greenland and at the species' southern limit range from Cadiz, Spain, to Rio Sado, Portugal. All samples were collected by diving except in Greenland, where in addition to diving, samples were also collected with an anchor dragged from a boat. From each population, 29 or more shoots were collected haphazardly but keeping at least 1–1.5 m distance between sampling units (except for Esteiro do Baião where shoots were collected at an interval of 0.5–1 m). Collected shoots were cleaned from salt and sediment and stored dry in silica crystals.

A second data set consists of six populations from the North Sea/Baltic region, which have been genotyped and published previously (Olsen *et al.* 2004). The sampling design of these populations was similar (Olsen *et al.* 2004), allowing the comparison of results between regions. The North Sea/Baltic region is considered the central diversity 'hotspot', corresponding to the mid-latitude along the species distribution. The same loci were used in both data sets to compare the results.

DNA extraction

Silica-dried leaves (2 cm per individual) were crushed by grinding for 30 s at 50 Hz (Retsch's Mixer Mill MM 300). DNA was extracted for 30 min at 60 °C using a slightly modified CTAB method (Doyle & Doyle 1988). The extracts were purified by two chloroform/isoamyl extractions (24:1) and precipitated with ethanol 100% using a standard protocol.

Microsatellite amplification and genotyping

Microsatellite development and primer sequences for the eight loci used can be found in Reusch *et al.* (1999) for loci ZosmarCT3, GA2 and GA6; and Reusch (2000a) for loci CT35, CT17H, CT19, CT20 and GA3; amplification protocols for multiplexing of loci and fragment

separation are given in Reusch *et al.* (2000). Fluorescently labelled PCR fragments were analysed on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) using the GeneScan-350 LIZ standard. Raw allele sizes were scored with STRAND (http://www.vgl.ucdavis.edu/informatics/strand.php), binned using the R package MsatAllele (Alberto 2009) in R software (R Development Core Team 2011) and manually reviewed for ambiguities.

Because seagrasses can propagate either clonally or sexually, we need to discriminate ramets (number of ramets = N, modular units of the same genetic individual) from genets (number of genets = G, the genetic individuals originating from distinct sexual recombination events, which can be composed of several ramets) distinguished based on their multilocus genotypes (MLG). Yet, a problem that must be addressed is that identical MLGs observed in two sampled ramets can correspond either to two ramets belonging to the same genet or to two different genets if, by chance, the sampled alleles are all identical between the two genets. The probability of encountering the latter depends on the population frequencies of the alleles observed in that genet and on the number of loci used to fingerprint samples (reviewed in Arnaud-Haond *et al.* 2007). To address this issue, we calculated the probability of a given MLG occurring repeated n times, as a consequence of different recombination events (P_{sex}) using the GenClone software (Arnaud-Haond & Belkhir 2007). Genotypic richness, the proportion of different genets in each sample, was estimated as by Dorken & Eckert (2001): R = (G−1)/(N−1).

Data analysis

After removal of clonal replicates, we calculated basic population genetic parameters for both data sets, that is, allele frequencies, alleles/locus, observed and expected heterozygosity (H_O/H_E), Hardy–Weinberg equilibrium, *F*-statistics and significance testing using the Genetix software (Belkhir *et al.* 1996–2004), and sequential Bonferroni was performed as a multiple test correction.

Allelic richness (Â) was estimated for each population separately, standardized to 20 individuals to account for differences in sample size, using the StandArich package (http://www.ccmar.ualg.pt/maree/software.php?soft=sarich) and R software version 2.9.0 (R Development Core Team (2011)). For comparison between the rear edge and the diversity hotspot, allelic richness was also calculated by pooling all populations at the southern limit and the same for all populations from the North Sea/Baltic region as mentioned in Olsen *et al.* (2004).

IBD (Slatkin 1993) under a two-dimensional stepping-stone model (Kimura & Weiss 1964) was tested in the southern edge populations and the North Sea/Baltic sites using the matrix correlation method of Mantel (Manley 1994) implemented in ibd 1.2 (Bohonak 2002). $F_{ST}/(1-F_{ST})$ was calculated in Genetix 4.02 using the θ estimator, and geographical distances were estimated as distance along coasts. Strength of the IBD relationship was determined with reduced major axis regression as implemented in the program IBDWS (Jensen *et al.* 2005).

We contrasted genetic differentiation between the southern limit region and the North Sea/Baltic by comparing the regressions of F_{ST} on geographical distance. Because the North Sea/Baltic populations were sampled over a broader geographical range than the southern Iberian populations, we also analysed pairwise population pairs within the same range of geographical distances (≤350 km). The Greenland populations were not analysed for IBD because of the limited number of populations and sample size per population after removal of clonal replicates.

A one-way analysis of co-variance (ancova) was performed to test for homogeneity of the regression lines, that is, testing the null hypotheses of no differences between the intercepts and between the slopes.

Results

The populations at the southern limit contained 376 genets out of 825 ramets genotyped. The 'leading' edge population (Greenland) revealed 51 genets out of 119 collected ramets. The centre of distribution showed 262 genets out of 371 ramets collected in six populations (Olsen *et al.* 2004). The estimated probabilities of identical MLG having been derived from independent reproductive events (P_{sex}) were <0.05, leading to the recognition of a total of 689 individual genets.

Genotypic richness (R) at the southern limit was highly variable between populations (Table 1), ranging from 0.09 (Sado) to more than 0.80 (Ponta do Adoche and Culatra). The leading edge populations in Greenland were also highly variable with R ranging from 0 to 0.82. In the North Sea/Baltic region, there was a clear difference between North Sea and Baltic populations, with high genotypic richness in the North Sea (R ranging from 0.97 to 1) where almost all shoots collected represented a unique individual genet, and medium genotypic richness in the two Baltic populations (0.41 and 0.44). Mean genotypic richness in the North Sea/Baltic region was significantly higher than in the south (northern mean $R = 0.705$, southern mean $R = 0.455$, t-test $P < 0.05$).

Mean expected heterozygosity (H_E) was significantly different between the three regions (anova, $F = 24.23$

Table 2 Matrix showing pairwise population F_{ST} values (above diagonal) per region and the distance in kilometres between populations per region (below diagonal) measured as the shortest distance along the coast

	Amer 1	Amer 3	Kapi					
Leading edge								
Amer 1	0	**0.066**	0.476					
Amer 3	14	0	0.454					
Kapi	173	187	0					
	Falk	Maas	SyltM	LangrN	HoogeT	Gron2		
Diversity hotspot								
Falk	0	0.02780	0.06205	0.13806	0.11579	0.21006		
Maas	70	0	0.08894	0.14711	0.13781	0.21553		
SyltM	870	870	0	0.03134	**0.00893**	0.09948		
LangrN	912	912	98	0	0.02959	0.08604		
HoogeT	958	958	142	51	0	0.08584		
Gron2	1103	1103	297	196	150	0		
	PA	Troia	VNM	Bar	PLS	EstB	Fuzeta	Cadiz
Rear edge								
PA	0	**0.00952**	0.18163	0.24780	0.26812	0.20364	0.25956	0.20067
Troia	2	0	0.18064	0.24504	0.25903	0.19970	0.25628	0.18865
VNM	100	100	0	0.22477	0.22438	0.23074	0.22395	0.22099
Bar	285	285	185	0	0.07438	0.23697	0.07718	0.21903
PLS	285	285	185	22	0	0.26510	0.06262	0.26978
EstB	285	285	185	8	20	0	0.28754	0.20698
Fuzeta	285	285	185	30	13	33	0	0.25273
Cadiz	445	445	325	160	160	160	160	0

F_{ST} values in bold are not significant. The Kobbe population in the leading edge was removed because of sample size. The same was done for the Sado and Arrábida populations in the rear edge (For site abbreviations, see Table 1).

$P = 0.0001$), and all pairwise comparisons were significant (Tukey's HSD test, $P < 0.05$). Heterozygosity was highest in populations from the North Sea/Baltic region (mean $H_E = 0.5769$; range, 0.4739–0.6658) than in the southern region (mean $H_E = 0.4270$; range, 0.3091–0.5515). Heterozygosity was lowest in Greenland (mean $H_E = 0.2398$; range, 0.1875–0.3223) (Table 1).

Significant positive F_{IS} (heterozygote deficiency) was more prevalent in the North Sea/Baltic region (four of the six populations) than in the southern region (three out of the nine populations, i.e. Ponta do Adoche, Tróia and Cadiz). Such heterozygote deficiency could be caused by inbreeding, Wahlund effect (pooling populations with different allele frequencies) or putative null alleles. Heterozygote excess (significant negative F_{IS}) was observed in only two populations at the southern edge (Sado and Barrinha, see Table 1) and may be due to selective advantage of individual clones (Billingham *et al.* 2003).

Populations in each region are highly structured as shown by high F_{ST} values. Almost all F_{ST} are highly significant ($P < 0.05$ after Bonferroni correction), except in the leading edge populations Amerillik 1 and 3, which are only 14 km apart (Table 2). Within the Ria Formosa, high F_{ST} values were found mainly for pairwise comparisons involving the Esteiro do Baião population. The F_{ST}'s between this population and the other sites within this lagoon are of the same order as F_{ST} between Ria Formosa and other southern limit sites (see Table 2). In the North Sea/Baltic region, populations are also structured, but F_{ST} are significantly lower than in the south (*t*-test $P < 0.0001$).

Distance across the rear edge is *c.* 500 km (Cadiz to Sado), which is half of the maximum distance sampled in the North Sea/Baltic part of the distribution (*c.* 1000 km between Groningen and Falkenstein). Genetic distance among populations, as measured by F_{ST}, correlated positively with distance within each region: the southern limit ($r = +0.3839$, Mantel's test $P = 0.037$) and the diversity hotspot ($r = 0.8046$, Mantel's test $P = 0.0079$) (Fig. 2, lines a and c). If we remove the long-distance pairwise comparisons to compare across similar ranges, the correlation is even better (Fig. 2, regression lines b and d). When pairwise comparisons within the same range of geographical distances are considered, the slopes of the regression

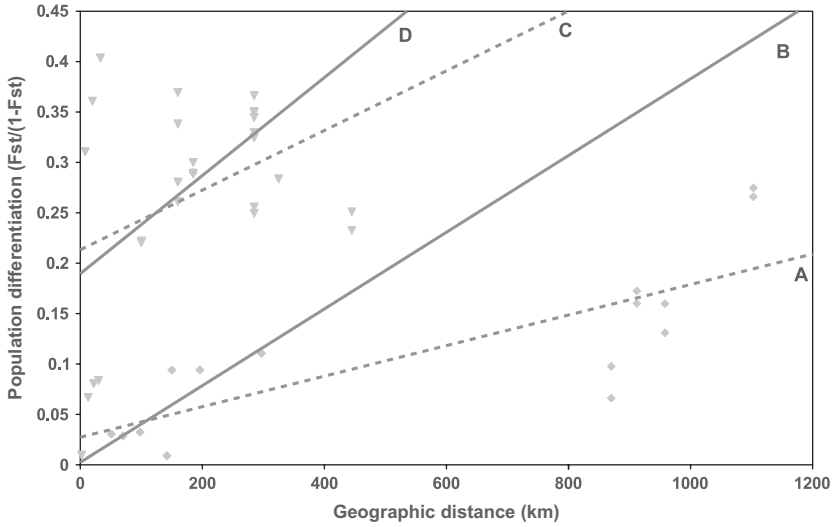

Fig. 2 Isolation by distance. Pairwise comparisons of genetic differentiation and geographical distance (kilometres) for the southern limit populations (triangles) and the North Sea/Baltic region (diamonds). For each region, two regression lines are shown. Dashed grey lines are regressions with all pairwise comparisons; solid grey lines are regressions using only pairwise comparisons within the same geographical distance range so that correlations could be compared. The genetic and geographical distance matrices were significantly correlated for both regions (southern limit, Mantel test; $P = 0.037$ and $r = 0.3839$, North Sea/Baltic, Mantel test; $P = 0.007$ and $r = 0.8046$). Slopes of regressions lines b and d were not significantly different (ANCOVA, $P = 0.808$), but the intercepts with the y-axis are significantly different (ANCOVA, $P < 0.0001$).

(Fig. 2, lines b and d) are not significantly different between the southern limit and the North Sea/Baltic region (ANCOVA: $F = 0.06$, $P = 0.808$). However, the intercepts are significantly different (ANCOVA: $F = 34.28$, $P < 0.0001$). Thus, differentiation increases with geographical distance at equal rates in the North Sea/Baltic populations and the southern populations, but populations in the latter are more genetically differentiated from each other at all distances (Fig. 2).

Allelic richness was standardized (Â) for 10 individuals per population for comparability except for those populations with <10 genets (Table 1). The two populations with <10 genets (Rio Sado and Arrábida) were left out of comparison of genotypic richness and standardized allelic diversity. Standardized Â varied considerably between populations but was significantly higher (t-test $P < 0.001$) in the centre populations (4.5–5.7) than in the 'rear' edge (2.5–4.2). The 'leading' edge showed the lowest allelic richness (1.3–2.4) (Fig. 3). When populations in each region are pooled, allelic richness (also standardized for 10 individuals for comparison) in the North Sea/Baltic range and at the southern limit is not significantly different (5.5 and 5.2, respectively; Fig. 3). Allelic richness in the leading edge is much lower (3.1), also when populations are pooled. When standardized for 50 individuals, which is possible after pooling populations, allelic richness remains very similar between North Sea/Baltic and southern regions; 9.58 (SE 0.431) for the North Sea/Baltic region and 9.25 (SE 0.412) for the southern limit (Fig. 3).

Discussion

Populations in southern Iberia (the limit of distribution) contain as much genetic variation as in the diversity 'hotspot' in the North Sea/Baltic region, contrary to inferences based on poor edge population sampling. This finding contradicts the major hypothesis that edge populations are genetically depleted and highlights the role of between-population diversity at range edges. Population diversity comparisons are commonly performed on diversity averages for individual populations, while our results show that pooling populations within a region is necessary to reveal the genetic diversity retained in the region. High regional genetic diversity in edge populations has been reported for terrestrial species (see Hampe & Petit 2005), but only few cases have recently been documented from the marine environment (Provan & Maggs 2011; Neiva *et al*. 2012).

We confirmed our hypothesis that populations of *Zostera marina* at the southern limit of distribution (Iberian Peninsula) show decreased genetic diversity within populations but increased genetic differentiation compared to populations from the diversity 'hotspot' in the northern range of the distribution. Our results show that populations from the 'rear' edge have lower intrapopulation allelic richness, heterozygosity and genotypic richness than populations from the North Sea/Baltic region. The northernmost 'leading' edge has the lowest genetic diversity [both allelic richness (Â) and

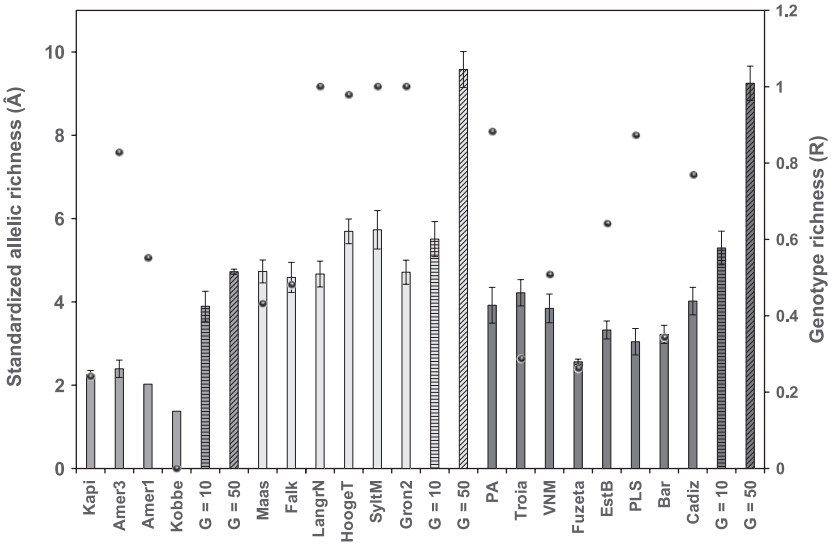

Fig. 3 Standardized allelic richness (left axis and bars) and genotypic richness (right axis and dots) for individual populations and for the pooled populations for each region. Different shades of grey represent different regions, and within each region are also shown the individual populations (solid bars), pooled populations with Â standardized for $G = 10$ individuals (horizontal stripes) and pooled populations with Â standardized for $G = 50$ individuals (hatched bars). Population abbreviations are explained in Table 1. Error bars are standard errors.

genotypic richness (R)]. Populations from both southern Iberia and North Sea/Baltic are highly structured, but differentiation in the latter was considerably lower, despite the distance between populations being twice as large compared to the south. The southern edge populations are small and isolated, and separated from other small populations by unsuitable habitat; therefore, high drift and low gene flow are the likely drivers of their higher genetic differentiation and lower genetic diversity.

The Iberian Peninsula was an important refuge for several marine organisms during the LGM (e.g. Hoarau *et al.* 2007; Provan & Maggs 2011; Neiva *et al.* 2012). Historical refuge areas are expected to have retained high genetic diversity compared to areas that have been recolonized after the LGM, thus subject to founder events. Although southern Iberia was likely a historical refuge for *Z. marina*, it now happens to be at the southern limit of its distribution, and to be highly endangered (Cunha *et al.* 2011). Edge populations are expected to undergo loss of genetic diversity because of drift associated with low effective population size; this is likely to be the case in *Z. marina*.

The unexpected highest genetic diversity in the North Sea/Baltic region (Olsen *et al.* 2004) was explained by two hypothetical scenarios: (i) a secondary contact zone where two expansion fronts meet, one from the southeast Atlantic and the other from the West Atlantic and (ii) the possibility that the North Sea/Baltic, because of sea surface currents from the south and the north running into it, is entraining rafting *Z. marina*, providing a continuous supply of new genotypes into the region. Under this second scenario, other species would be expected to have been affected by the same current flow processes resulting in similar patterns, but this is unknown for other species. Whichever the source of new genotypes in this area, present conditions favour persistence of high diversity. Populations in this region show high gene flow, sexual reproduction predominating over clonal and high levels of out-crossing (Reusch 2000b, Reusch 2001, 2002, 2003; Olsen *et al.* 2004), maintaining high genetic variation across the region.

Southern Iberia tells a different story. Where once extensive meadows existed along the south Iberia coast (Cunha *et al.* 2011), the region underwent a strong decline in seagrass cover during the past few decades, and although no single major cause is known, interactive effects of heavy winter storms and run-off, increased fish grazing and various anthropogenic actions have surely contributed to the decline (Cunha *et al.* 2011). Southern populations nowadays consist of few very small meadows separated by large unsuitable habitat, and some of the populations sampled for this study have since gone extinct (Cunha *et al.* 2011). This isolation and reduction in population size may have been the major cause for the loss of genetic diversity at the population level as small isolated populations diverge quickly because of low gene flow and high drift. Dominance of clonal reproduction at the edges further accentuates this effect. This pattern of low intrapopulation diversity leads to apparent rejections of the predictions of high diversity in past refugial zones. However, when considering the whole southern region (pooling all populations), there is considerably more genetic diversity, at a level comparable to the North Sea/Baltic diversity hotspot. Pooling of populations in the south leads to a twofold increase in allelic richness, while in the North Sea/Baltic, pooling of populations does not increase Â. This means that genetic diversity in this more northern region is generally similar among

populations, whereas in the south, each population is more unique and contributes differently to the overall allelic richness.

The Greenland leading edge populations show the lowest Â, even after pooling. This is expected for populations found in formerly glaciated areas (Hewitt 1996, 2004). The North Sea/Baltic region was also a glaciated area, but more recent effects (listed above) might contribute to its current richness. Other hypotheses for the low diversity in the Greenland area are possible, namely bottlenecks in periglacial refugia, which may mimic the low diversity patterns resulting from recolonization from southern refugia (Brochmann *et al.* 2003).

Our finding has important implications for conservation issues. The high genetic diversity found in the North Sea/Baltic diversity hotspot was considered of extreme importance for conservation objectives and was proposed as a model for monitoring biodiversity in relation to climate change (Olsen *et al.* 2004). We argue that the rear edge should also be considered as an important diversity hotspot because it still maintains high genetic diversity for monitoring biodiversity in relation to climate change but in a different way and for different reasons.

Rear edge populations are typically small and restricted to particular habitat islands within a matrix of unsuitable landscapes (Hampe & Petit 2005). These populations have persisted for longer time periods in relative isolation, which resulted in reduced within-population genetic diversity (Castric & Bernatchez 2003; Petit *et al.* 2003). Their isolated life results in extremely high population differentiations even at small geographical distances, which leads to extraordinary levels of regional genetic diversity (Comps *et al.* 2001; Castric & Bernatchez 2003; Hampe *et al.* 2003; Petit *et al.* 2003; Martin & McKay 2004).

In rear edge populations, selection for local adaptation to their environment is suggested to play an important role (Dynesius & Jansson 2000), which may result in the development of distinct ecotypes (Castric & Bernatchez 2003). However, low genetic diversity in such marginal populations may prevent local adaptation (Pearson *et al.* 2009). In experimental inter-population crosses (Billingham *et al.* 2007), southern edge *Z. marina* populations showed both inbreeding depression when mating within populations as well as outbreeding depression when mating across distances of only tens of kilometres, from the open sea to a very enclosed marshy site (Esteiro do Baião), a highly differentiated population (see Results) occupying a distinct ecological niche. This suggests that despite low diversity, local adaptation was still possible in a recent past. This pattern of outbreeding depression among small isolated populations in the south indicates that pooling distinct populations for conservation purposes (e.g. in restoration) may decrease their offspring fitness despite increasing genetic diversity. If large-scale restoration is the goal, it would then be preferable to select single donor populations that contain high genetic diversity, when attempting to minimize both inbreeding and outbreeding depression, whereas populations from the rear edge would not be appropriate donors under this criterion. However, it is still important to conserve their regional diversity because such locally adapted genotypes hold important evolutionary potential in face of future environmental change.

Rear edge populations have an important storage function for genetic diversity. They may be extremely important for the persistence and evolution of the species, particularly as new selective pressures arise with environmental changes. It is therefore important to conserve these rear edges in such a way as to maximize the number of local populations regardless of their size and performance. This is different from the general idea of conserving the viable core populations (Hampe & Petit 2005).

Acknowledgements

We thank Jeanine Olsen for kindly providing the *Z. marina* data set from the North Sea/Baltic. We are grateful to the following people for help in field sampling or providing samples: Ed Morris (Cadiz), Martin Billingham (Ria Formosa), the Biomares team (Tróia), the ATP team: Peter Bondo Christensen, Dorte Krause-Jensen, Birgit Olesen and Nuria Marbá (Greenland). This study was supported by a postdoctoral fellowship to OED from the FCT-Portuguese Science Foundation, Portugal, and by research projects PPCDT/MAR/60044/2004 (FCT-FEDER), SHIFTING (EU-MarinERA–CTM2008-04183-E/MAR) and ATP (Arctic Tipping Points, FP7-ENV-2008-1-226248).

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Data accessibility

Information on sampling location and microsatellite data for the Baltic/North Sea diversity 'hotspot' can be found in Olsen *et al.* 2004.

Microsatellite data: DRYAD entry doi:10.5061/dryad.2589rn16.