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North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L.

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Abstract

As the most widespread seagrass in temperate waters of the Northern Hemisphere, *Zostera marina* provides a unique opportunity to investigate the extent to which the historical legacy of the last glacial maximum (LGM18 000–10 000 years BP) is detectable in modern population genetic structure. We used sequences from the nuclear rDNA–internal transcribed spacer (ITS) and chloroplast *mat*K-intron, and nine microsatellite loci to survey 49 populations (> 2000 individuals) from throughout the species' range. Minimal sequence variation between Pacific and Atlantic populations combined with biogeographical groupings derived from the microsatellite data, suggest that the *trans*-Arctic connection is currently open. The east Pacific and west Atlantic are more connected than either is to the east Atlantic. Allelic richness was almost two-fold higher in the Pacific. Populations from putative Atlantic refugia now represent the southern edges of the distribution and are not genetically diverse. Unexpectedly, the highest allelic diversity was observed in the North Sea–Wadden Sea–southwest Baltic region. Except for the Mediterranean and Black Seas, significant isolation-by-distance was found from ~150 to 5000 km. A transition from weak to strong isolation-by-distance occurred at ~150 km among northern European populations suggesting this scale as the natural limit for dispersal within the metapopulation. Links between historical and contemporary processes are discussed in terms of the projected effects of climate change on coastal marine plants. The identification of a high genetic diversity hotspot in Northern Europe provides a basis for restoration decisions.

Keywords: Ice Age, ITS, microsatellites, phylogeography, seagrass, *Zostera marina*

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Introduction

The effects of the last glacial maximum (LGM 18 000–10 000 years BP) have profoundly influenced virtually all shallow-water, coastal marine habitats in the North Atlantic. Glacial melt, retraction of ice sheets and changes in sea level led to a concomitant range expansion of taxa from southern Atlantic refugia into newly opened areas and/or from refugia in the Pacific. The effects of the LGM have been extensively studied in the northern European terrestrial flora and fauna (reviewed in Hewitt 2000). However, a

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similar effort for benthic marine species throughout the North Atlantic has only recently gained momentum (Cunningham & Collins 1998; Avise 2000; CORONA 2002).

A number of pioneering studies in the early 1990s clearly established the *trans*-Arctic pathway (Dunton 1992; Crame 1993) with connections both predating (Vermeij 1991; molluscs) and postdating (Palumbi & Kessing 1991; sea urchins) the opening of the Bering Strait at 6.4 Ma based on the most recent estimates (Marincovich & Gladenkov 1999). Although the emphasis has clearly been on invertebrates (Wares & Cunningham 2001; Luttikhuizen *et al*. 2003), seaweeds (van Oppen *et al*. 1994, 1995; Lindstrom *et al*. 1996; Coyer *et al*. 2003) and seagrasses (Reusch *et al*. 2000; Coyer *et al*. 2003) have also been investigated.

Consideration of marine seagrasses and seaweeds is important because the contemporary North Atlantic marine fauna is generally considered to be primarily of Pacific origin; whereas the North Atlantic marine flora has traditionally been assumed to be primarily of Atlantic origin (Garbary & South 1990). Dunton (1992) dubbed this generalization the 'paradox of the *trans*-Arctic exchange' and attributed the lower intrusion rate between the Atlantic and Pacific to poor dispersal capacities, unfavourable current regimes, lack of sufficiently hard substrata and ice scouring, which would have affected seaweeds more than other benthic faunal assemblages. It has also been assumed that the presence of a richer endemic marine flora in the North Pacific empirically demonstrates the putatively restricted interchange (e.g. Garbary & South 1990; Lüning 1990) between the Pacific and Atlantic. However, in a recent review of the Arctic marine flora, Lindstrom (2001) concluded that 85–100% and 55–80% is shared with the North Atlantic and North Pacific, respectively, and argued that marine floral exchanges have occurred with greater frequency from both directions than previously supposed.

Eelgrass (*Zostera marina* L.) is the most widely distributed seagrass in temperate waters of both Pacific and Atlantic coasts in the Northern Hemisphere. Like all seagrasses, it is highly productive, provides habitat for diverse animal assemblages and helps to stabilize coastal erosion (Hemminga & Duarte 2000; Bruno & Bertness 2001; Williams & Heck 2001). Recognition of the continuing worldwide loss of seagrasses (~15% over the past decade) due to both natural and anthropogenic causes (Short & Wyllie-Echeverria 1996; Green & Short 2003), has raised questions of long-term viability related to possible genetic erosion, inbreeding depression, lower fitness and limited recovery capacity (see Williams 2001). Climate change is also expected to affect present distributions of temperate seagrasses (Short & Neckles 1999). Along European shores the ranges of many species are expected to retreat 300–500 km northward within the next century as a consequence of rising sea surface temperatures (IPPC 2001). Related to this, changes in the North Atlantic Oscillation are predicted to cause more rainfall with subsequent dilution of already brackish Baltic habitats causing a southward shift of species already living at their salinity limits.

In this study, we survey regional to oceanic-scale population genetic structure. We focus on two general questions:

1 How has recent climatic history shaped the distribution of *Z. marina* in the North Atlantic?
2 To what extent are historical imprints of refugia and recolonization detectable in the current global pattern of differentiation in relation to geographical distance?

The first question is approached from two perspectives. We first assess intraspecific phylogenetic relationships in *Z. marina*, based on nuclear ribosomal DNA–internal transcribed spacer (rDNA–ITS) and chloroplast *mat*K-intron sequences. A deep divergence between Pacific and Atlantic populations would support a Pleistocene (6 Ma) or even earlier Tethyan (10–12 Ma) separation. In contrast, a shallow divergence would support a recent connection following the opening of the Bering Strait (between 6.4 and 3.5 Ma), and very little or no divergence would suggest contemporary exchange within the resolution limits of ITS. In a parallel approach, we looked for genetic footprints of northward expansion from southern refugia in the Atlantic using population-level diversity displayed by microsatellite DNA polymorphisms. The simplest model hypothesizes a southern refuge along one or both sides of the Atlantic roughly corresponding to 41° N (New York) on the western side and 43° N (northern Spain) on the eastern side, which denote the southern ice limits (Frenzel *et al*. 1992). Based on sea surface temperature (SST) reconstructions (CLIMAP 1984), these refugial coastlines may have extended southward to, respectively, 37° N (Virginia, USA) and 20° N (Mauritania). Theory predicts that refugial areas will harbour the highest genetic diversity with attenuation northward 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). Likewise, younger, northern populations are predicted to be less structured than older southerly populations. This pattern has been repeatedly found in European terrestrial biota (Hewitt 2000, 2001) and, most recently in the seaweed, *Fucus serratus* (Coyer *et al*. 2003). However, this effect can be counteracted by postglacial bottlenecks or extinction/recolonization events thus reducing genetic diversity at putative glacial refugia that are presently near the species ecophysiological tolerance limits (e.g. Coyer *et al*. 2003).

We approached the second general question by examining pairwise population differentiation: first, in terms of a neighbour-joining (NJ) analysis of genetic distance in order to establish patterns of natural disjunctions; and second, to delimit interpopulation connectivity under a model of isolation-by-distance (IBD).

Materials and methods

Sample collection and processing

Samples were collected from 49 locations across the Northern Hemisphere (Table 1). At each location 25–100 shoots were collected at 1–1.5 m intervals (except for a few at a distance of 0.5–1 m at Baiao, Portugal, because of restricted meadow size) using a haphazard walk or swim. Clean pieces of 2–3 connected leaves were blotted dry and placed on silica drying crystals. Details of DNA extraction are given in Reusch *et al*. (1999c).

rDNA–ITS and matK-intron amplification and sequencing

Primer sequences used to amplify both of the ITS of the nuclear rDNA region as a single amplicon were forward: 5′-GGAAGTAAAAGTCGTAACAA-3′ and reverse: 5′-GCATCGATGAAGAACGCAG-3′. The chloroplast-encoded *mat*K-intron was amplified using forward: 5′-TCAAATT-CTACAATGCTGGA-3′ and reverse: 5′-GGTTGAGACC-AAAAGTRAAAAT-3′. A 10-μL PCR reaction contained: 1–2 μL template DNA (for ITS) or 5 μL (*mat*K) of a Sephaglas resuspension, 10 mM Tris–HCl, 50 mM KCl, 0.1% Triton X-100, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μM of each primer (0.5 μL of 20 μM stock), 0.01% BSA and 0.05 U *Taq* DNA polymerase (Promega). Polymerase chain reactions (PCR) were performed with a PTC-100 (MJ Research) thermocycler, with the following amplification profile: 94 °C for 3 min followed by 30 cycles of 94 °C for 1 min, annealing at 42 °C (for ITS) and 55 °C (for *mat*K), and extension at 72 °C for 2 min. PCR products were directly sequenced using Big Dye Terminator Cycle Sequencing (Applied Biosystems) and separated on an ABI 377 GeneAnalyser (Applied Biosystems).

Microsatellite amplification and fragment separation

Microsatellite development and primer sequences for the nine loci used can be found in Reusch *et al*. (1999c) for loci *ZosmarCT3*, *GA2* and *GA6*; and Reusch (2000a) for loci *CT35*, *CT17H*, *CT12*, *CT19*, *CT20 and GA3*; amplification protocols for multiplexing of loci and fragment separation are given in Reusch *et al*. (2000). Amplification products were separated on an ABI 377 automated sequencer (Applied Biosystems) and analysed using GENESCAN software (Applied Biosystems).

Identification of genets and clonal diversity

As for all seagrasses, *Zostera marina* is characterized by a modular structure of genets (= the genetic individual) and ramets (= leaf shoots belonging to a genet) (*sensu* Harper 1977). Because the probability that a nine-locus, microsatellite genotype will be found more than once is extremely low (Reusch *et al*. 1999b), the presence of identical multilocus genotypes provides a convenient way to identify clones of the same genetic individual. Clonal diversity (*C*) was determined by dividing the number of genets by the number of ramets sampled. Although highly dependent on the sampling design and size, this index provides meaningful comparisons across stands sampled in the same way. Identical multilocus genotypes were removed from the data for all further analyses.

Total data set and core data set

We used two versions of the data set: one including all 49 populations, allowing for some missing loci and variable sample sizes; and a 'core' set consisting of 22–24 populations (shown in italics in Tables 1 and 2) from across the biogeographical range, with no missing loci and uniform sample sizes.

Sequence analyses

DNA sequences were managed and aligned using BIOEDIT 5.0.9 (Hall 1999). Phylogenetic analysis and bootstrap resampling (1000 replicates) were performed using maximum parsimony under the exhaustive search option in PAUP* 4.0b.10 (Swofford 2002). Given the simplicity of the data set, likelihood and Bayesian methods were not necessary.

Microsatellite analysis

Basic data on the genetic composition (allele frequencies, alleles/locus, heterozygosity, Hardy–Weinberg equilibrum [HWE]), *F*-statistics and significance testing were calculated using GENETIX (Belkhir *et al*. 2001).

Potential saturation of the loci for detecting genetic distance was tested by calculating the maximum-predicted-multiple-allelic estimate of F_{ST} following the method of Jin & Chakraborty (1995) as derived in Hedrick (1999) following Equation 2a. This was necessary because of the naturally fast mutation rate of microsatellite loci and the uncertain stepwise mutation model, which can result in overlapping sets of alleles due to homoplasy in completely isolated populations (Nauta & Weissing 1996).

Allelic richness (*A*) was estimated using rarefaction in the program CONTRIB (Petit *et al*. 1996) because sample sizes (following correction for duplicate ramets) were often unequal. Rarefaction uses the frequency distribution of alleles at a locus to estimate the number of alleles that would occur in smaller samples of individuals and allows for an unbiased estimate of allelic richness (Leberg 2002). In *Z. marina*, a rarefaction to $N = 20$ (40 gene copies) or $N = 40$ (80 gene copies) was used for a core set of populations.

Table 1 Sample collection data and habitat characteristics for *Zostera marina*. Locations in italics are part of the core set of populations used in some calculations (see Materials and methods). The geographical locations of the sampling are shown on the maps in Figs 5–7

Location	Code	Position	Collector	*N*	Ref.	GenBank ITS-1/ITS-2 *mat*K	Depth (m)	Annual/ Perennial
C Baltic Sea–Finland								
1, Henriksberg, Finland	Henrik	59°50′ N, 23°09′ E	C. Boström	32			2.5	p
2, Plagen, Finland	Plagen	59°49′ N, 22°59′ E	C. Boström	31			3.0	p
3, Ryssholm, Finland	Ryss	59°60′ N, 22°59′ E	C. Boström	31			1.4–3	p
4, Prästö, Åland Is.	Prasto	60°10′ N, 20°32′ E	C. Boström	31			1.4–4	p
5, Sandö, Åland Is.	Sando	60°17′ N, 20°23′ E	C. Boström	33			4.3	p
W Baltic Sea–Kiel Bight–Kattegat St.								
6, Möltenort, Germany	Molt	54°03′ N, 10°13′ E	T. Reusch	42	†		1.8–2.6	p
7, Falkenstein, Germany	Falken	54°24′ N, 10°12′ E	T. Reusch	80	*,†		2–3	p
8, Kiekut, Germany	Kiek	54°09′ N, 09°52′ E	T. Reusch	34	†		2–2.6	p
9, Wackerballig, Germany	Wack	54°46′ N, 09°53′ E	T. Reusch	53	†		1–1.5	p
10, Maasholm, Germany	Maas	54°41′ N, 10°00′ E	T. Reusch	110	*,†		1–1.5	p
Wadden Sea–North Sea								
11, Konigshafen, Sylt, Germany	SyltKon	55°03′ N, 08°25′ E	T. Reusch	27	*,†		0.2	p/a
12, Leghorn, Sylt, Germany	SyltLeg	54°58′ N, 08°22′ E	T. Reusch	26	*,†		intertidal	a
13, Munkmarsch, Sylt, Germany	SyltMunk	54°54′ N, 08°22′ E	T. Reusch	48	†		‘	‘
14, Rantum, Sylt, Germany	SyltRat	54°51′ N, 08°19′ E	T. Reusch	33	†		‘	‘
15, Langeness, Germany (Priel)	LangN	54°39′ N, 08°32′ E	T. Reusch	35	†		‘	‘
16, Langeness, Germany (Watt)	LangW	54°39′ N, 08°03′ E	T. Reusch	28	†		0.2–0.6	p
17, Hooge, Germany (Priel)	HoogePr	54°34′ N, 08°32′ E	T. Reusch	46	†		0.2–0.6	p
18, Hooge, Germany (tidal)	HoogeT	54°32′ N, 08°01′ E	T. Reusch	48	†		intertidal	p/a
19, Groningen, Netherlands (Dollard, Paap2)	Paap2Gr	53°20′ N, 06°00′ E	J. Olsen & W. Stam	50		AY553590 AY553601 AY551313	intertidal	p/a
E Atlantic coast–Europe								
20, Ålesund, Norway	AlsundN	63°30′ N, 07° E	H. Christie	50	‡		10	p
21, Lambhusatjorn, Iceland	LambIce	64°22′ N, 22° W	J. Coyer	50	‡	AY553589 AY553600 AY551312	3–4	p
22, Arnarnesbogur, Iceland	ArnaIce	64°22′ N, 23° W	J. Coyer	30			3–4	p
23, Old Grimsby, Scilly I. (Cornwall)	Grimsby	49°57′ N, 06°19′ W	S. Widdicombe	50			1–2	p
24, Carantec, France (Brittany)	Carantec	48°45′ N, 03°45′ W	M.-P. Oudot-Le Secq	37	‡	AY553591 AY553602 AY551314	1	p
25, Morgat, France (Brittany)	MorgatB	48°11′ N, 04°33′ W	T. Reusch	40	*		1	p
26, Ria Formosa, Portugal (Esteiro do Baiao)	BAPort	36°50′ N, 07°40′ E	M. Billingham	39			0.2–0.5	p
27, Ria Formosa, Portugal (Ponta da Culatra)	PLPort	36°50′ N, 07°45′ E	M. Billingham	47			0.2–0.5	p
Mediterranean Sea								
28, Étang de Thau, France	Thau	43°25′ N, 03°36′ E	T. Reusch	40	*		1.8–2.5	p (lagoon)

Table 1 *Continued*

Location	Code	Position	Collector	*N*	Ref.	GenBank ITS-1/ITS-2 *mat*K	Depth (m)	Annual/ Perennial
Black Sea–Azov Sea								
29, Sukhoy Liman, Ukraine	Sukoy	46°03′ N, 30°20′ E	V. Alexandrov	50	‡	AY553592 AY553603 AY551315	1–2	p (lagoon)
30, Odessa City Beach, Ukraine	Odessa	46°20′ N, 30°10′ E	V. Alexandrov	50			1–2	p (lagoon)
31, Sevastopol, Ukraine	Sevast	44°05′ N, 33°45′ E	V. Alexandrov	50			1–2	p
32, Kazachya Bay, Ukraine	Kaza	44°05′ N, 33°50′ E	N. Milchakova	50			1–2	p (lagoon)
33, Cape Fonar, Kersch Strait	Fonar	45°10′ N, 36°30′ E	N. Milchakova	50			2–3	p
34, Molochny coastal salt lake, Azov Sea	Molo	46°40′ N, 35°30′ E	N. Milchakova	50			3	p
35, Utlyuk coastal salt lake, Azov Sea	Utlyuk	46°25′ N, 35°20′ E	N. Milchakova	50			3	p
W Atlantic coast–USA–Canada								
36, Halifax, Nova Scotia	Halifax	44°42′ N, 63°11′ W	T. Reusch	38	*‡	AY553587 AY553598 AY551310	0.2–0.4	p
37, Ninigrit Pond, Narragansett Bay, RI	Ninigrit	41°21′ N, 71°38′ W	S. Granger	50			1	p (lagoon)
38, Rose I., Narragansett Bay, RI	Rose	41°29′ N, 71°20′ W	S. Granger	50	‡	AY553588 AY553599 AY551311	1	p
39, Woods Hole, MA	Woods	41°31′ N, 70°40′ W	A. Govindarajan	50			1–2	p
40, St. James Bay, Quebec	JamesQ	53°00′ N, 79° – ′W	R. Lumière	50			1–2	p
E Pacific coast–USA–Canada								
41, Auke Bay (near Juneau), AK	Auke	58°19′ N, 134°23′ W	E. Calvert	50	‡	AY553593 AY553604 AY551316	1	p
42, Samish I., Padilla Bay (sub), Puget Sound, WA	PadillaS	48°34′ N, 122°32′ W	S. Wyllie-Echeverria	50	‡	AY553594 AY553605 AY551317	1.5	p
43, Samish I., Padilla Bay (inter), Puget Sound, WA	PadillaI	48°34′ N, 122°32′ W	S. Wyllie-Echeverria	50	‡		intertidal	p
44, South Beach, Shaw I., Puget Sound, WA	Shaw	48°33′ N, 122°56′ W	S. Wyllie-Echeverria	50	‡		1.5	p
45, Bodega Channel, Bodega, CA	Bodega	38°30′ N, 123°05′ W	J. Olsen & W. Stam	50	‡	AY553595 AY553606 AY551318	inter	p
46, Smuggler's Cove, Santa Cruz I., Channel Is., CA	CruzS	34°01′ N, 119°40′ W	J. Coyer	25	‡	AY553596 AY553607 AY551319	3–4	p
47, Prisoner's Cover, Santa Cruz I., Channel Is., CA	CruzP	34°01′ N, 119°30′ W	J. Coyer	25	‡	AY553597 AY553608 AY551320	3–4	p
48, Coast Guard Beach, San Nicolas I., Channel Is, CA	Nicol	33°15′ N, 119°30′ W	J. Coyer	20			10	p
49, Big Geiger, Santa Catalina I., Channel Is., CA	Catalina	33°28′ N, 118°31′ W	T. Reusch	30	*		3–5	p

*Reusch *et al*. (2000).
†Reusch (2002).
‡Used in rDNA–ITS and *mat*K-intron sequencing comparisons.

Table 2 Summary of multilocus genetic variation for all populations of *Zostera marina* (Table 1) based on nine microsatellite loci. Localities printed in italics belong to the core populations used in some calculations. N = sample size (following removal of duplicate ramets from the same genet); N_A = number of alleles; H_E = unbiased expected heterozygosity; H_O = observed heterozygosity; f = inbreeding coefficient and F_{ST} = global variance component contributed by an individual locus. Significant values (bold) after sequential Bonferroni correction (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). An expanded version of this table giving the per locus values is available as supplementary material from the corresponding author

	C. Baltic–Finland					Baltic–Kiel Bight–Kattegat					Wadden Sea	
	1 Henrik	*2 Plagen*	*3 Ryss*	*4 Prasto*	*5 Sando*	*6 Molt*	*7 Falken*	*8 Kiek*	*9 Maas*	*10 Wack*	*11 SyltKon*	*12 SyltLe*
Total (N)	16	22	12	12	12	37	39	32	48	50	25	26
Private/total N per region		0/74				3/206					3/308	
Mean H_E	0.3356	0.4444	0.4195	0.1708	0.2273	0.5142	0.4756	0.4924	0.4399	0.4568	0.6337	0.6213
Mean H_O	0.3899	0.4954	0.4167	0.213	0.3687	0.4889	0.45	0.4848	0.389	0.4477	0.6271	0.5673
Multilocus f	−0.16788	−0.11818	0.00702	**−0.2625*****	**−0.6743*****	0.04987	0.05463	0.01556	**0.1166*****	0.01999	0.01059	0.08852
	Wadden Sea–North Sea							E Atlantic coast–Europe				
	13 SyltMunk	*14 SyltRat*	*15 LangN*	*16 LangW*	*17 HoogePr*	*18 HoogeT*	*19 Paap2*	*20 AlsundN*	*21 LambIce*	*22 ArnaIce*	*23 Grimsb*	*24 Car*
Total (N)	48	33	34	28	46	47	50	34	31	29	14	20
Private/total N per region								1/214				
Mean H_E	0.6747	0.6438	0.6349	0.5932	0.6879	0.6401	0.5405	0.4292	0.3246	0.3204	0.5825	0.4951
Mean H_O	0.6153	0.5922	0.5609	0.562	0.6529	0.613	0.4548	0.4301	0.2652	0.2835	0.5529	0.5293
Multilocus f	**0.08898*****	0.08146	**0.1180*****	0.05349	0.05145	0.04277	**0.1598*****	−0.00221	0.18547	0.11679	0.05266	−0.07096
	E. Atlantic–Europe			Med.	Black Sea–Azov Sea							
	25 *Morgat*	26 BAPort	27 *PLPort*	28 *Thau*	29 Sukhoy	30 Odessa	31 Sevast	32 Kazach	33 Fonar	34 Molo	35 Utlyuk	36 Halifax
Total (N)	36	18	32	32	42	6	7	49	31	23	9	34
Private/total N per region				2/31	8/167							
Mean H_E	0.4699	0.3416	0.3719	0.5447	0.3463	0.3899	0.4469	0.4084	0.3758	0.4131	0.2709	0.5318
Mean H_O	0.4533	0.4259	0.4309	0.5188	0.3333	0.3333	0.4603	0.3539	0.362	0.4106	0.2716	0.5278
Multilocus f	0.03588	**−0.25589**	**−0.16171**	0.04832	0.0379	**0.1666*****	−0.03264	**0.01345*****	0.03733	0.00611	−0.00285	0.00769
	W. Atlantic coast–USA–Canada				E. Pacific coast–USA–Canada							
	37 *Ninigrit*	38 *Rose*	39 *Woods*	40 *JamesQ*	41 Auke	42 PadillaS	43 *PadillaI*	44 Shaw	45 *Bodega*	46 CruzS	48 Nicol	49 Catlina
Total (N)	35	49	45	50	29	33	50	10	48	21	3	1
Private/total N per region		3/213					13/177 (all Puget Sound and Bodega)					
Mean H_E	0.5496	0.5633	0.5215	0.4101	0.3088	0.4885	0.4465	0.4632	0.606	0.4009	0.15	—
Mean H_O	0.6	0.5125	0.4214	0.3911	0.2804	0.4719	0.3939	0.4875	0.509	0.3359	0.1667	—
Multilocus f	**−0.0932*****	**0.09111****	**0.1936*****	0.04665	0.0693	0.03446	**0.1188*****	−0.05564	**0.1609*****	**0.1641*****	−0.14286	—

	Total N_A	Global F_{ST}/locus	Expected Max F_{ST} (core pops)
GA2	19	0.215	0.462
GA23	17	0.360	0.514
GA35	42	0.075	0.220
GA17H	44	0.038	0.149
GA12	20	0.576	0.484
GA19	15	0.681	0.698
GA20	27	0.527	0.640
GADU	27	0.513	0.574
GA16	21	0.618	0.639
Total (N)	**1438**	—	
Total alleles	**232**		
multilocus F_{ST} (all)	0.397		

Biogeographical relatedness among populations was analysed using pairwise Reynold's as well as Cavalli-Sforza distances computed from the microsatellite allele frequencies followed by a NJ analysis and bootstrap resampling (1000 replicates). These analyses were performed using GENDIST, NEIGHBOUR, SEQBOOT and CONSENS in the program package PHYLIP 3.5 (Felsenstein 1994).

IBD (Slatkin 1993) under a two-dimensional stepping-stone model (Kimura & Weiss 1964) was tested using the matrix correlation method of Mantel (Manley 1994) implemented in IBD 1.2 (Bohonak 2002). $F_{ST}/1 - F_{ST}$ (estimated as $\theta/1 - \theta$) was calculated in GENETIX 4.02 and geographical distances were estimated either along coasts or using great circle distances. Strength of the IBD relationship was determined with reduced major axis (RMA) regression as implemented in the program IBD.

Results

Sequence data and intraspecific phylogeny

Based on rDNA–ITS and *mat*K-intron sequences, *Zostera marina* is the basal species within the genus *Zostera* (Fig. 1). Sequence variation within the final alignment (883 bp, no gaps) was extremely low. ITS1 (222 bp) was invariant; two positions varied in ITS2 (239 bp) and four positions varied in the *mat*K-intron (422 bp) (Fig. 1B). Only two informative positions separate the east Pacific from the Atlantic-*sensu lato*. No variation was detected among Atlantic isolates, which ranged from the west Atlantic to the Black Sea. Separation of *Z. marina* populations north and south of Point Conception, CA (a major biogeographical boundary separating the cooler northern and warmer southern marine biotas; Fig. 1C), as well as the failure of two microsatellite loci to amplify the southern ones, suggests high population differentiation, possibly at the level of nascent speciation.

Microsatellite data quality and properties

More than 2000 ramets were genotyped with nine microsatellite loci revealing a total of 232 alleles and 1400 genets (Tables 1–3) A sample size of 20 genets was sufficient to capture the diversity of the loci based on the variation in mean number of alleles/locus plotted against increasing

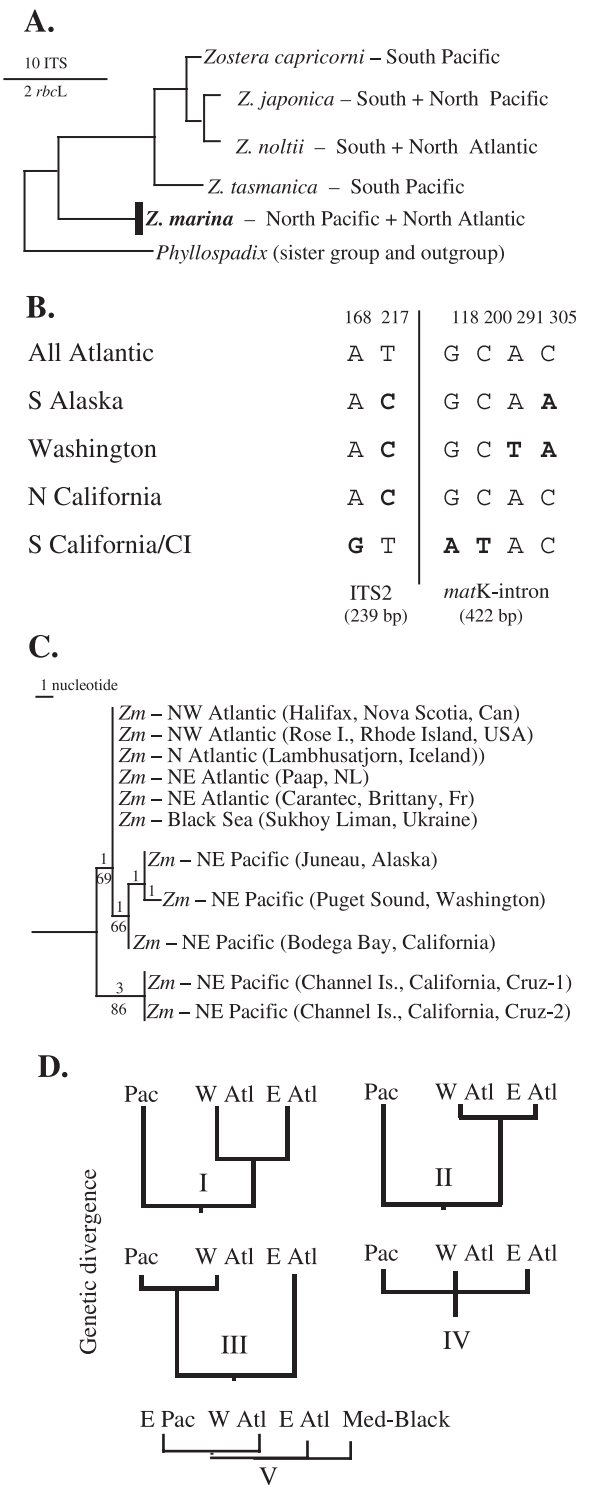

Fig. 1 Phylogenetic relationships within *Zostera* based on DNA sequence data. (A) Among-species relationships based on nuclear rDNA–ITS and chloroplast-encoded *rbc*L sequences redrawn from Les *et al*. (2002). Branch lengths are proportional to nucleotide differences. All clades have > 95% bootstrap support. (B) Mini-alignment of the six variable positions found among 884 nucleotides of the combined alignment. Variable sites are shown in bold. Numbers above columns refer to nucleotide positions in the original alignment. (C) Within-species relationships for *Z. marina* based on a combined analysis of ITS and *mat*K-intron sequences. Branch lengths are proportional to nucleotide differences. Bootstrap values are given below. (D) Alternative hypotheses of colonization history (modified from Cunningham & Collins 1998 in which branch lengths are proportional to divergence time. See also Avis 2000). *Zostera marina* is a Type IV or V. See text.

Table 3 Clonal diversity and allelic richness in *Zostera marina* from Table 1. Clonal diversity (*C*) was expressed as a function of the number of ramets sampled and the number of genets detected based on all nine loci (spatial scale of the sampling was always 1–1.5 m between samples). Bold values of $C < 0.50$ indicate the presence of a few large clones in the area. Allelic richness (*A*) was calculated for 22 core populations based on rarefaction (sample size 40) and six loci (73 alleles) following Petit *et al*. (1996)

Population	No. ramets sampled	No. genets determined	Clonal diversity (*C*)	Genets with > 1 ramet	Ramets per genet	Allelic richness rarefaction (*A*)
C Baltic Sea–Finland						$\bar{x}$ = 1.17
Henrik1	32	9	**0.28**	5	1-16	
Plagen2	31	15*	**0.48**	8	1-5	1.17
Ryss3	31	12	**0.38**	5	1-7	
Prasto4	31	6	**0.19**	5	1-17	
Sando5	33	4	**0.12**	4	1-15	
W Baltic Sea–Kiel Bight–Kattegat						$\bar{x}$ = 4.06
Mölt6	42	37	0.88	3	1-3	
Falken7	80	62	0.77	11	1-6	3.60
Kiek8	34	32	0.94	2	1-2	
Wack9	53	50	0.94	3	1-2	
Maas10	110	48	**0.43**	27	1-9	4.53
Wadden Sea–North Sea						$\bar{x}$ = 4.10
SyltKon11	27	25	0.92	1	2	
SyltLeg12	26	26	1.00	0	1	
SyltMunk13	48	48	1.00	0	1	5.18
SyltRat14	33	33	1.00	0	1	
LangN15	35	35	1.00	0	1	3.35
LangW16	28	28	1.00	0	1	
HoogeP17	46	46	1.00	0	1	
HoogeT18	48	47	0.98	1	2	5.13
Paap2Gr19	50	50	1.00	0	1	2.75
E Atlantic coast						$\bar{x}$ = 1.82
AlsundN20	50	34	0.68	11	2-4	2.69
LambIce21	50	31	0.62	10	2-3	1.51
ArnaIce22	30	29	0.97	8	2-7	1.64
Grimsby23	50	16	**0.32**	13	2-6	
Carantec24	37	20	0.54	7	2-59	
MorgatB25	40	36	0.90	3	2-3	1.80
BAPort26	39	18	**0.46**	11	2-5	1.49
PL Port27	47	32	0.80	10	2-5	
Mediterranean Sea						$\bar{x}$ = 3.16
Thau28	40	39	0.97	1	2	3.16
Black Sea–Azov Sea						$\bar{x}$ = 2.18
Sukoy29	50	39	0.78	6	2-3	1.70
Odessa30	50	6	**0.12**	3	2-42	
Sevast31	50	7	**0.14**	14	2-6	
Kaza32	50	48	0.96	3	2	2.66
Fonar33	50	9	**0.18**	11	2-4	
Molo34	50	23	0.46	11	2-4	
Utlyuk35	50	9	**0.18**	13	4-23	
W Atlantic coast–USA–Canada						$\bar{x}$ = 3.92
Halifax36	38	36	0.95	1	2	4.88
Ninigrit37	50	35	0.70	9	2-7	3.53
Rose38	50	49	0.98	1	2	4.74
Woods39	50	45	0.90	4	2	3.56
JamesQ40	50	50	1.00	0	1	2.91
E Pacific coast–USA–Canada						$\bar{x}$ = 5.56
Auke41	50	29	0.58	10	2-8	
PadillaS42	50	33	0.66	10	2-5	6.38
PadillaI43	50	50	1.00	0	1	4.91
Shaw44	50	10	**0.20**	8	2-9	
Bodega45	50	48	0.96	1	2	6.38
CruzS46	25	21	0.84	3	2	
CruzP47	25	11	**0.44**	6	2-9	
Nicol48	20	3	**0.15**	3	3-8	
Catalina49	30	1	**0.03**	—	30	

*15 genets were allowed for the allelic richness calculation.

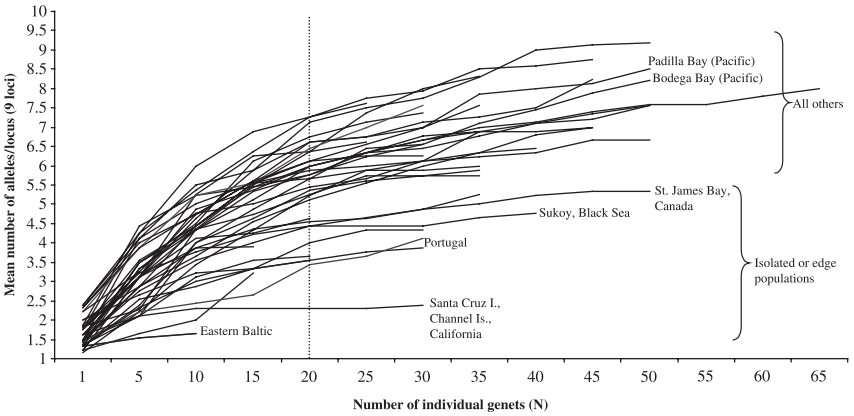

Fig. 2 Variation of the mean number of alleles per microsatellite locus with population sample size (following removal of duplicate ramets from the same genet) for *Zostera marina*. Each line represents one population.

sample size (Fig. 2). With the exception of the Plagen, Finland population, all sample sizes used in subsequent calculations were well above this threshold. The total number of alleles per locus ranged from 17 to 44. Loci *CT35* and *CT20* failed to amplify in several Pacific populations. Almost all loci proved to be fixed in one or more populations. However, some regions (north Baltic, Black and Azov Seas, California Channel Isands) had exceptionally high proportions of fixed alleles (An expanded form of Table 2 including results per locus is available as supplementary material from the corresponding author).

A global observed F_{ST} was calculated for each locus over all individuals (Table 2) in order to gain some insight into each locus's contribution to the variance. Loci *CT35* and *CT17H* contributed little, which was expected given that very polymorphic loci have smaller expected F_{ST} values even under complete population separation (Hedrick 1999). The remaining seven loci contributed fairly equally to the global F_{ST}.

The core set of populations (Tables 1–3), composed of 864 individuals and 186 alleles represented ~80% of the total number of alleles across the full biogeographical range (except for the California Channel Isands) with almost no loss in variance (global F_{ST} = 0.397, core–global F_{ST} = 0.385).

Clonal diversity

Clonal diversity varied widely (Table 3). The vast majority of duplicate genotypes involved 2–3 adjacently sampled individuals, which corresponded to an average-minimum genet size of 2–4 m in any direction. The largest clones were associated with the north Baltic (> 50 m), southern Portugal (≈6–10 m), several locations in the Black and Azov Seas (> 75 m), Shaw I, WA (> 20 m) and in the California Channel Islands (> 50 m). Large clones were not correlated with latitude or with allelic richness, highlighting the importance of local factors in determining genetic structure.

Allelic richness

No consistent correlation was detected between mean allelic richness and latitude (Fig. 3A). When sea surface temperature (SST) minima and maxima were substituted for latitude, winter SST revealed a shift of the Skagererrak/Kattegat population from Norway towards the warmer temperatures (arrow, Fig. 3A), whereas summer SST maxima showed a shift in the north Baltic populations towards warmer temperatures.

Mean allelic richness was also examined in sequentially smaller partitions of the data set (Fig. 3B). In the largest-scale partition (east Pacific, Atlantic and Mediterranean–Black–Azov Seas), the allelic richness in the east Pacific was nearly twice as high as that found in the Atlantic. The Atlantic, in turn had a slightly higher diversity than the Mediterranean–Black–Azov Seas region. In a second partition of west and east Atlantic, the west Atlantic displayed a higher allelic richness, due either to a link with the Pacific or as an artefact of greater sampling effort in the east Atlantic. The highest diversities occurred at the same latitudes in both the east Pacific and west Atlantic. In the smallest partition of the data (regions within the east Atlantic-*sensu lato*), allelic richness was unexpectedly distributed in a unimodal pattern, with the highest diversity in the Wadden Sea and Kattegat region of the southwest Baltic. A subpartitioning of allelic richness within the east Pacific data set was problematic because of inadequate sample sizes, nonamplificable loci and/or fixed loci making a direct comparison with Fig. 3 impossible. In a separate set of calculations we used seven loci and three populations spanning 3000 km. In this case, a clear south-to-north decrease in allelic richness (mean, SE) was observed from Bodega Bay, CA (10.2, 1.2) to Padilla Bay, WA (8.0, 0.6) and Auke Bay, AK (5.4, 2.3).

Population differentiation

Virtually all of the 1176 pairwise estimates of F_{ST} were highly significant. The exceptions occurred among highly

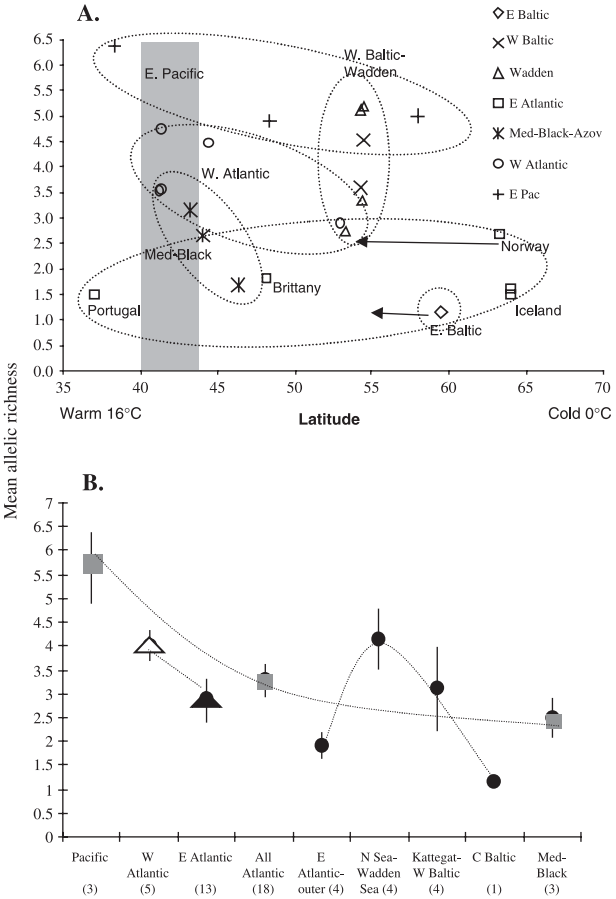

Fig. 3 Mean allelic richness based on the core populations following rarefaction. (A) Mean allelic richness vs. latitude. Circled areas indicate biogeographical regions; the grey bar indicates the maximum northern latitude during the LGM. (B) Mean allelic richness per region in which each point represents the multilocus mean and standard error of the pooled populations designated along the *x*-axis. Connected squares show the trend among pooled Pacific, Atlantic and Mediterranean–Black–Azov Sea populations (numbers in parentheses); connected triangles compare western and eastern Atlantic populations in which the filled triangle represents the mean of the circle populations for comparison. The connected circles compare subregions within the eastern Atlantic. Connecting lines are a guide for the eye and have no statistical meaning.

connected populations in the German Wadden Sea and southwest Baltic previously analysed by Reusch (2002). The minimum scale of significant differentiation was 5 km in the central Baltic, whereas the maximum distance of no differentiation was 75 km between Möltenort and Wackerballig in the southwest Baltic.

The expected maximal mulitlocus F_{ST} for all nine loci was 0.484; excluding *CT17H*, 0.522; and excluding *CT35* and *CT17H*, 0.585 (Table 2). In all but a few cases (Portugal, Thau Lagoon, Black Sea), pairwise F_{ST} values were mostly < 0.350, indicating that observed divergences are not due to marker saturation. In the aforementioned exceptions, F_{ST} values were > 0.500. Populations from these areas were

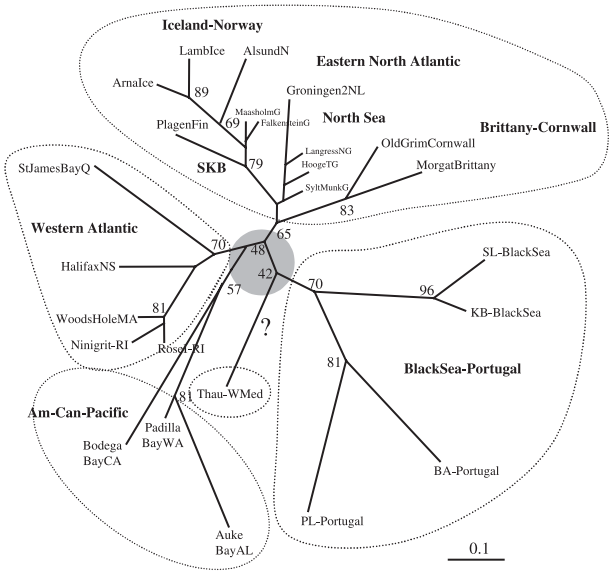

Fig. 4 Neighbour-joining tree based on Reynold's distances derived from the microsatellite data for *Zostera marina*. Low bootstrap support among the major biogeographical regions is indicated by the shaded circle at the centre. This analysis was based on eight loci (*ZosmarCT35* omitted). Circled clades were analysed separately with more populations and/or different loci in Figs 5–7.

characterized by low clonal diversity (probable small N_e) and isolation leading to rapid genetic drift and high differentiation (Billingham *et al*. 2003).

Large-scale biogeographical groups

The NJ analysis emphasized the closeness among distant oceanic groupings (Fig. 4). The east Atlantic–Baltic clade was well resolved with three sub-clades corresponding to the Brittany–Cornwall area, the North Sea–Wadden Sea, and the Skagerrak–Baltic Sea. Icelandic populations were associated with Norway and not with North America or the Pacific. A west Atlantic group consisted of a Cape Cod cluster with connections to Halifax (~500 km distant) and St. James Bay, Quebec (~3500–4000 km distant). The three Pacific populations from Auke Bay, AK (southern Alaska), Puget Sound, WA and Bodega Bay, CA consistently grouped with the west Atlantic clade. Populations from the Black Sea formed a well-supported clade as did the two populations from the Ria Formosa in southern Portugal. The western Mediterranean population from Thau Lagoon always grouped with Portugal and the Black Sea despite the lack of bootstrap support. Attempts to improve the resolution of the central part of the tree using Cavalli–Sforza chord distances did not alter the topology, internodal distances or levels of bootstrap support. Selective removal of populations with long branches (e.g. Portuguese populations) also had no effect, nor did the addition of more Pacific populations (using fewer loci).

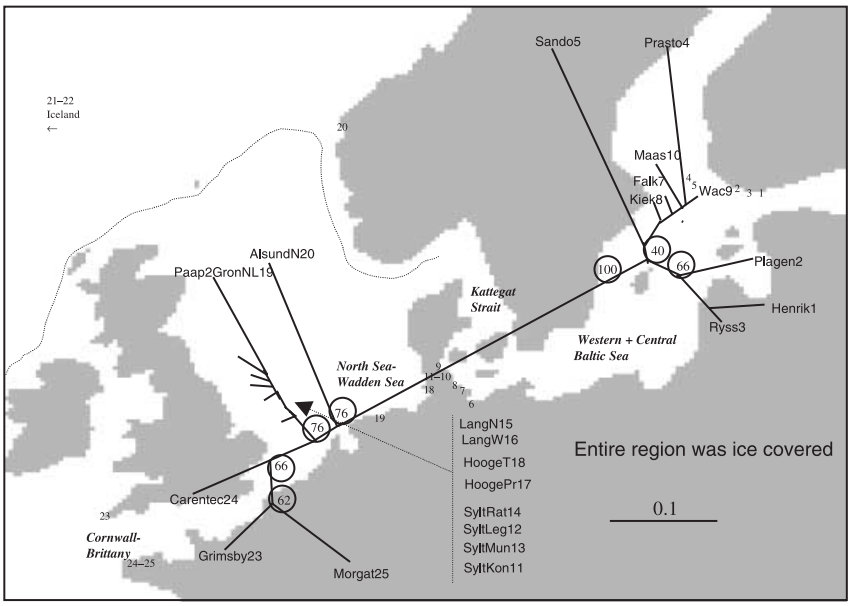

Fig. 5 Neighbour-joining tree based on Reynold's distances from microsatellite loci among eastern Atlantic populations of *Zostera marina*. Circled numbers along branches are bootstrap values. Smaller numbers associated with coastal areas correspond to place names in Table 1. Dotted line indicates the 200-m contour of continental shelf.

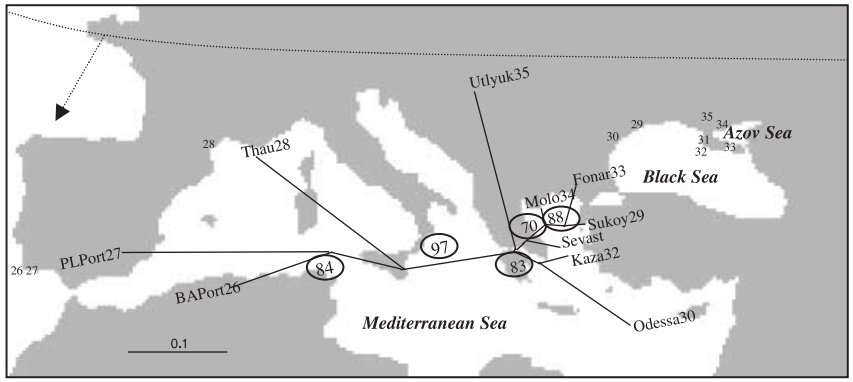

Fig. 6 Neighbour-joining tree based on Reynold's distances from microsatellite loci among Mediterranean, Black and Azov Sea populations of *Zostera marina*. Circled numbers along branches are bootstrap values. Smaller numbers along coastal areas correspond to place names in Table 1. Dotted line indicates approximate edge of the ice during the LGM with a possible extension as far south as the northwest tip of the Iberian Peninsula.

Each of the four major clusters was analysed in more detail with additional populations and different combinations of loci. All Baltic populations were strongly separated from the North Sea, Wadden Sea and Atlantic region (Fig. 5 and eight loci, *GA6* excluded; sample sizes as shown in Table 3). The Cornwall–Brittany area was associated with the English Channel and North Sea.

The Mediterranean group (all loci, sample sizes as shown in Table 3), revealed a clear separation between the Black–Azov Sea populations, and the populations from Thau Lagoon in the west Mediterranean and the Ria Formosa in southern Portugal (Fig. 6). Even though the Portuguese populations are geographically part of the Atlantic coast, they did not group with the north-coast Brittany populations. There are no records of *Z. marina* from north Spain and the only populations currently existing along the Portuguese coast are extremely small and all occur south of the Tagus River, an important biogeographical boundary for marine populations (e.g. Diekmann *et al*. submitted).

The east Pacific and west Atlantic (seven loci, *CT35*, and *CT20* excluded; sample sizes as shown in Table 3) analysis showed a moderate divergence between the California Channel Islands (just south of Point Conception, a biogeographical boundary) and all other regions (Fig. 7). The divergence was also found in the phylogenetic analysis (Fig. 1). Populations from northern California, Puget Sound, WA and Auke Bay, AK formed a tight cluster with the west Atlantic although internal bootstrap support was poor. The unexpected association of the Bodega Bay sample with Halifax may reflect an introduction. Significantly, Bodega Bay was the only Pacific population in which *CT35* amplified.

Isolation-by-distance

Population groupings for IBD analysis were based on the initial results of the NJ analysis. The southwest and north Baltic differed substantially in population differentiation and IBD. In the southwest Baltic, IBD was not present

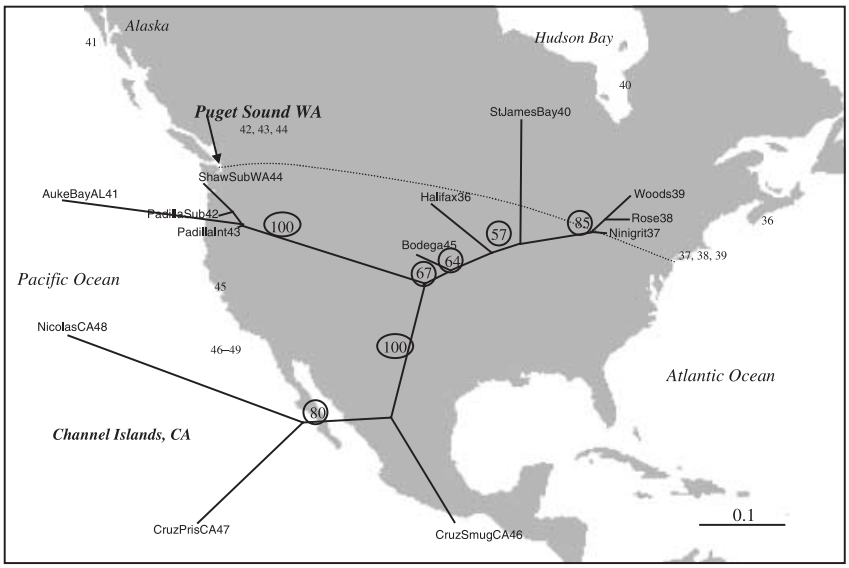

Fig. 7 Neighbour-joining tree based on Reynold's distances from microsatellite loci among North American populations of *Zostera marina*. Circled numbers along branches are bootstrap values. Smaller numbers along coastal areas correspond to place names in Table 1. Dotted line indicates the approximate edge of the ice during the LGM.

< 75 km, but central Baltic populations were 10–50 times more differentiated with steep and significant IBD over the same spatial scale (Fig. 8A,B). Dutch and German Wadden Sea populations were intermediate to the Baltic populations with weak differentiation and significant IBD between 10 and 250 km (Fig. 8C). Following the results from the NJ analysis in Fig. 4, the Norwegian and Icelandic populations were added in order to extend the spatial range to 4000 km (Fig. 8D). Although IBD was very strong, a break point at ~150 km was evident. The addition of more long-distance populations (Fig. 8D) further magnified the break point, indicating the probable natural limits of dispersal on a regional scale. Populations from the west Atlantic (Fig. 8D) showed IBD comparable with that found for the Wadden Sea–Norway–Iceland group. Sampling was insufficient to determine whether a similar 150-km break point exists for the west Atlantic. Contrary to expectation, no IBD was observed between the Mediterranean and Black Sea regions (Fig. 8F).

The east Pacific populations (excluding the California Channel Islands) showed strong population differentiation and significant IBD but only 28% of the variation was explained (Fig. 8E). The addition of more east Pacific populations, particularly from within Puget Sound and the Queen Charlotte Islands (areas likely to facilitate strong local differentiation because of their many islands) may produce a range of local differentiation values, at comparable distances, similar to those observed for the east Atlantic in Fig. 8(E).

Discussion

Pacific origins and intraspecific phylogenetic connections with the Atlantic — how recent is recent?

Modern seagrasses are believed to have evolved during the last 30 Ma (Larkum & den Hartog 1989) and the highest diversity of both genera and species is found in tropical to subtropical waters of the Indo-West Pacific (e.g. Hemminga & Duarte 2000). The Zosteraceae is the most dominant temperate family and is monophyletic with five species of *Zostera* (Fig. 1A) based on a phylogenetic analysis using *rbc*L, *trn*K and rDNA–ITS sequences (Les *et al*. 2002). The most basal and widely distributed species of the family, *Zostera marina*, diverges in ITS sequence by 16% from its sister species. Based on an ITS clock for green plants and algae of 0.8–2%/Ma (Bakker *et al*. 1995), *Z. marina* originated in the Pacific between 8 and 20 Ma.

Connection between the Pacific and Arctic Oceans has occurred at least twice since the late Miocene: at ~17 Ma (Sher 1999) and again, beginning ~6.4 Ma (Marincovich & Gladenkov 1999). Although today's currents flow from west to east through the Bering Strait, prior to the closure of the Isthmus of Panama (~3.5 Ma), they flowed from east to west, which would have created a barrier to Atlantic dispersal prior to that time. Thus, the opportunity for *Z. marina* to radiate into the Atlantic occurred only within the past 3.5 Ma. If Pacific and Atlantic populations had remained isolated, as has been shown for numerous other Pacific–Atlantic biota (Avise 2000), then we predict an ITS sequence divergence of at least 2–6% and perhaps as much as 12% between oceans. This was clearly not the case, as the near absence of sequence variation (Fig. 1C) indicates an extremely recent divergence time. Furthermore, the absence of any ITS sequence variation within the Atlantic basin-*sensu lato*, including the Mediterranean and Black Seas, rules out the possibility of a Tethyan (Rögl & Steininger 1984) relict population of *Z. marina*, as suggested for the Mediterranean seagrass *Posidonia oceanica* (Procaccini *et al*. 2001) based on its disjunction with Australia. Thus, among the four phylogeographical hypotheses recognized by Cunningham & Collins (1998), *Z. marina* represents a Class

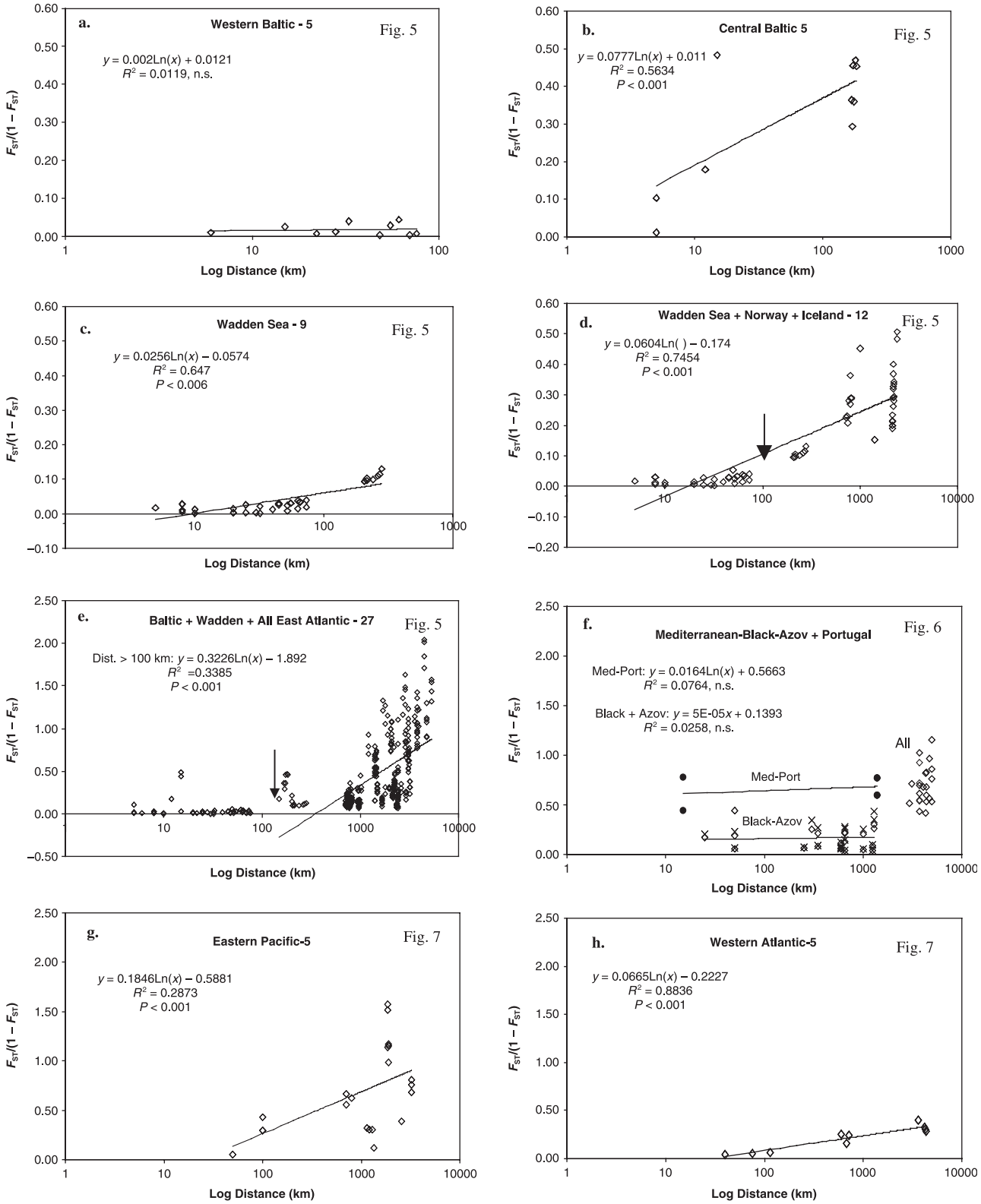

Fig. 8 Isolation-by-distance for *Zostera marina*. (A–D) The main Northern European subregions; (E) all European together in which the arrow marks a break point at ~150 km after which a sharp increase in IBD occurs; (F) Mediterranean–Black and Azov Sea regions; (G) eastern Pacific from the Channel Islands, California to Juneau Alaska; (H) western Atlantic from New Hampshire to Halifax. Note differences in *y*-axis scaling.

IV pattern (recent genetic connections between the east Pacific, west and east Atlantic, Fig. 1D). Even within this timeframe, however, repeated glacial–interglacial episodes have caused several cycles of extinction–recolonization of which the LGM is only the most recent example.

The NJ tree based on microsatellite distance data (Fig. 4) recovered several well-resolved clades, all of which were very closely connected. Even though bootstrap support was poor, the east Pacific group consistently linked with the west Atlantic group (see also Fig. 7) and never with the east Atlantic. Even if the Pacific populations were eliminated from the analysis (not shown), two well-supported west and east Atlantic clades remained. These results also point to the recency of differentiation, perhaps on the order of only a few thousand generations. We have depicted this as a new Class V in Fig. 1(D) in which the *trans*-Arctic exchange is still active — as is rapid differentiation within the North Atlantic itself. An underestimation of the actual divergence, particularly among distantly related populations due to saturation, was ruled out, as all of our values between the Pacific and Atlantic link are well below the maximum expected F_{ST}.

Contemporary gene flow between Pacific and Atlantic populations of *Z. marina* is quite feasible in our opinion. Rare, long-distance dispersal has been documented in marine seaweeds (van den Hoek 1987) and the fact that *Z. marina* has moderately buoyant leaves and spathes, durable seeds (Orth *et al*. 1994) and a monoecious mating system, make gradual, stepping stone dispersal a viable possibility. Using genetic assignment tests, Reusch (2002) was able to document this type of dispersal for up to 54 km. Second, parts of the Beaufort Sea along the northern Alaskan–Canadian coastline (including some of the island archipelagos of the northern Canadian Arctic) are open during the short summer season (ARO Chapman, personal communication). An active Pacific–Atlantic link may therefore be empirically demonstrated with extended sampling of the high Arctic (> 70° N). Third, the possibility of rare, successful dispersal by migratory birds cannot be ruled out (Nacken & Reise 2000). Finally, one should not assume that an 8000–10 000 km passage from the North Pacific into the Atlantic would require unrealistic amounts of time. For example, 29 000 plastic bathtub toys that were lost from a container ship at 45° N in transit between China and Seattle reached the Bering Strait within 3 years and Iceland within 5 years (Ebbesmeyer 2003). Thus, on the timeframe of the past several thousand years, opportunities for successful long-distance dispersal — perhaps requiring many decades of stepping stone advancement — have probably occurred thousands of times.

Allelic richness, refugia and diversity hotspots in the east Atlantic

A general comparison of allelic richness across the east Pacific, west and east Atlantic (Fig. 3B), clearly shows a decrease in the Atlantic as compared with the Pacific, which is consistent with the evolution of *Z. marina* in the Pacific and the fact that much of the Pacific region remained ice-free at higher latitudes, providing more extensive refugia during the LGM. Within the east Atlantic, however, the predicted northward decrease in allelic richness in support of an expansion from southern refugia could not be demonstrated (Fig. 3A). This finding was initially unexpected because previous studies focusing on recolonization pathways in the fucoid seaweeds *Fucus serratus* (Coyer *et al*. 2003) and *Ascophyllum nodosum* (Olsen, unpublished) (both with similar thermal optima to *Z. marina*), identified the Brittany Peninsula as part of the refugial, high-diversity centre from which a southern and northern attenuation in allelic richness could be clearly shown. Today, only remnant populations of *Z. marina* are found along the southern coast of Portugal, the northwest Mediterranean and the Black Sea region (which did not yet exist during the LGM); and these are now genetically impoverished as a consequence of high temperatures, patchiness and isolation — all characteristics of boundary populations.

Surprisingly, the highest genetic diversity in *Z. marina* was found in the Wadden Sea–North Sea–southwest Baltic region (Fig. 3B). Given that these areas could only have been recolonized within the past 7500 years, the colonization process must have been rapid. Extensive rapid dispersal of broadly distributed species with large population sizes could result in a wholesale range shift that would not produce a bottleneck and the predicted latitudinal gradient of decreasing genetic diversity so frequently encountered (see Comps *et al*. 2001). Broadly distributed species are also likely to have found more refugia. Both of the aforementioned fucoid species, for example, are restricted to the Atlantic, whereas *Z. marina* has had opportunities for recolonization from both Atlantic and Pacific refugia. The confluence of several populations into the same 'new Atlantic' area of shallow, secondary contact could result in a higher diversity in northern populations.

Although it may at first seem odd that the Wadden Sea–North Sea–west Baltic region would be a genetic diversity hotspot for European *Z. marina*, historical records dating to the Middle Ages show that vast *Z. marina* meadows dominated the Dutch, German and Scandinavian coastlines (Boström *et al*. 2003). In these areas and elsewhere, beach wrack was routinely used for animal fodder, insulation and roofing (Wyllie-Echeverria & Cox 1999) well into the early 20th century. The contraction of aeral cover observed in this region today is the direct result of anthropogenic nutrient loading and habitat modification (Baden *et al*. 2003; Boström *et al*. 2003), as well as the mass mortality due to the slime mould-mediated 'wasting disease' in the 1930s (Muehlstein *et al*. 1991). Nevertheless, this modern bottleneck has not been strong enough to affect the genetic diversity. Significantly, the Wadden Sea–North Sea region has

also been found to be the centre of diversity for *Z. noltii* (dwarf eelgrass), a predominantly intertidal species whose range mirrors that of *Z. marina* along European coastlines (Coyer *et al*. submitted). Though speculative, the northeast-flowing current into the North Sea Basin via the English Channel, combined with the deep southward flowing current along the southern Norwegian coast into the Skagerrak-Kattegat region may entrain rafting *Z. marina*, thereby providing a constant supply of new genotypes to the area. Our study and those of Reusch (2000b, 2001, 2002, 2003) have clearly demonstrated high gene flow, high levels of outcrossing and predominantly sexual reproduction in much of this area. Whatever the cause and maintenance of this high diversity hotspot for *Z. marina*, we consider this an extremely important finding with respect to seagrass conservation objectives and as a model for monitoring biodiversity in relation to climate change.

Does clonality have a biogeographical signature?

Clonal diversity greatly affects local population structure, population fitness (Reusch 2001; Hämmerli & Reusch 2002, 2003a,b) and ultimately, metapopulation dynamics. For example, extreme clonality will lead to inbreeding, potentially (though not necessarily) lower fitness and lower dispersal potential. From the biogeographical perspective, however, the effects of clonal diversity are more difficult to generalize. Large clones were not correlated with latitude or allelic richness. However, lower clonal diversity was associated with one or more of the following: (i) physical isolation from nearest sampled population, especially old populations/clones such as those in the Åland Archipelago of the north Baltic (Reusch *et al*. 1999a); (ii) apparent stress associated with low salinity (e.g. central Baltic, Black Sea), depth-related light or dispersal limitation (Padilla Bay, WA; California Channel Islands), lack of water motion (e.g. semilagoons, atidal areas); or (iii) geographical location at the edge of the maximal thermal distributional range (e.g. southern Portugal, Thau Lagoon in the northwest Mediterranean) (Billingham *et al*. 2003).

Gene flow and isolation-by-distance — are there any generalizations?

Models of IBD are widely used because they account for the common observation that dispersal capabilities of many species are limited in most habitats. Though far from perfect (Whitlock & McCauley 1999), IBD provides a baseline for comparison across various spatial scales and regions that may provide clues about the relative importance of clonal diversity, gene flow and drift against other factors. Our findings for *Z. marina* (Fig. 8) confirms earlier conclusions, that IBD is region specific (Ruckelshaus 1996, 1998; Williams & Davis 1996; Williams & Orth 1998; Reusch *et al*. 2000; Reusch 2002), but additionally provide a set of IBD comparisons over a much larger range of scales and clonal diversities. Although IBD graphs are presented for the east Pacific (Fig. 8G) and west Atlantic (Fig. 8H), the discussion focuses on the east Atlantic where sampling coverage was much more extensive. Strong population differentiation does not always translate into IBD (reviewed in Bohonak 1999), especially in areas where gene flow and genetic drift are not in equilibrium (Hutchinson & Templeton 1999). For example, the central Baltic (Fig. 8B) and the Black Sea (Fig. 8F) are characterized by populations with low clonal diversity and high population differentiation, but with opposite effects on IBD over similar spatial scales. The Åland Archipelago in the central Baltic is one of the most island-rich archipelagos in the world with over 31 460 islands and small skerries (Granö & Roto 1991). These both isolate and connect neighbouring subpopulations in the form of stepping-stones. In contrast, no such stepping-stones are present in the Black Sea or Mediterranean Sea. In these areas, populations are small, isolated in lagoons, under strong physiological stress (salinity and temperature) and under the strong influence of genetic drift. Likewise, a particular spatial scale does not necessarily predict the level of population differentiation because both physical (e.g. influence of local current regimes) and biological (e.g. a mixed mating system) factors may play additive or antagonistic roles. This is illustrated by the contrast between the central and west Baltic (Fig. 8A,B). In the central Baltic, populations are isolated, clonal and highly differentiated over very short distances; whereas southwest Baltic (Fig. 8A) and Wadden Sea (Fig. 8C) populations are clonally diverse and relatively undifferentiated. Awareness of these regional differences, as well as the spatial scales over which gene flow is shaping the genetic neighbourhood of the metapopulation (*sensu* Husband & Barrett 1994), is crucial because it provides a guideline for the prediction of long-term viability in relation to possible habitat fragmentation. In northern Europe, this scale appears to be at ~150 km (Fig. 8D,E). Therefore, the maintenance of gene flow on the scale of tens of km can be taken into consideration when linking population genetic studies to restoration, as well as in determining the necessary size requirements for marine protected areas.

The fate of Z. marina *in Europe?*

Historical processes related to extinction and recolonizations have several temporal scales. Here we have mainly considered the background effects of millennial timescales of recolonization of the entire north Atlantic basin over the past 15 000 years. However, other events are known to have affected extinction and recolonization of *Z. marina* in the North Atlantic on decadal to centennial scales. Long-term

monitoring of *Z. marina* beds over the past century clearly point to fluctuations in areal cover. Along the Brittany coast of France, declines have been noted since the 1980s, which have been attributed to elevated sea surface temperatures (Glémarec *et al.* 1997). Similar observations apply to the Dutch and German coasts, Danish fjords and the Baltic (Boström *et al.* 2003). Collective observations over widespread areas clearly indicate that expansion and contraction of seagrass meadows track temperature change (Short & Neckles 1999; Hemminga & Duarte 2000; Frederiksen *et al.* 2004).

Zosterah marina has an extremely broad temperature tolerance range of 0–40 °C with optima between 5 and 20 °C (Fonseca *et al.* 1998 and references therein). Although broad temperature tolerance argues for flexibility and resilience of *Z. marina*, the extreme temperatures can only be withstood for relatively short periods. In general, areas experiencing summer temperatures > 20 °C are considered distributional boundary zones, such as southern Portugal, the northwest Mediterranean and parts of the Black, Baltic and Azov Seas. Even so, average temperatures within or just outside the optima may be uninformative, as *sustained* high temperatures exceeding some threshold may trigger the chain of events leading to die-off. Sustained high temperatures fall in two categories: those that occur over decadal scales and those that occur over weeks.

In the decadal timeframe, Rasmussen (1973) looked for correlations between sustained warm summer temperatures in combination with mild winters in Danish fjords and found that die-offs occurred when the number of consecutive days with water temperatures > 20 °C doubled; for example, from 10 °C (between 1900 and 1930) to 20 °C (between 1932 and 1951). Increases in Gulf Stream temperatures during that period also correlated with the mass mortality of *Z. marina* and *Z. noltii* attributed to the slime-mould-mediated 'wasting disease' that struck in the 1930s. Similar reports have been made for Cheaspeake Bay, west Atlantic side (see Cronin *et al.* 2003).

In the timeframe of weeks, Greve *et al.* (2003) have shown that at temperatures > 25 °C, respiration rapidly exceeded photosynthesis; and by 30 °C, the meristematic oxygen levels in *Z. marina* were anoxic. Although summer temperatures in Danish fjords *average* 17–20 °C, it is common for higher temperatures to persist for short periods. Greve *et al.* (2003) demonstrated that meristematic anoxia may play a key role in seagrass die-off because of the importance of the meristem to plant growth and the rapidity with which anoxia can occur. Thus, even in northern latitudes, shallow coastal waters and lagoons may experience critical threshold temperatures.

The contraction of *Z. marina* populations in northern Europe over the last 70 years, combined with projections of increasing sea surface temperatures and storm frequency under climate change scenarios, do not seem to bode well for *Z. marina*. Clearly, populations living at their physiological boundaries and/or as small, geographically isolated populations are more vulnerable because reduced genetic diversity, reduced sexual reproduction and inbreeding generally lead to lower fitness. However, inbreeding can be quite advantageous if populations are locally adapted, and local adaptation tends to select for inbreeding mating systems simultaneously, as observed in the seagrass *Cymodocea* (Serrão *et al.* unpublished). In general, however, such populations are at greater risk and likely to go extinct if local conditions shift too rapidly. However, all is not bleak. The reservoir of genetic diversity available to *Z. marina* in northern European populations, combined with interpopulational connectivity via gene flow at subregional scales, bodes well for ecological opportunity and the potential for rapid adaptation that can and will track climate change. Gene flow may actually enhance local population adaptation under shifting environmental gradients (Trussel & Etter 2001) by the arrival of new, potentially preadapted genotypes. Moreover, increased storm frequency (at least within limits) may well enhance population connectivity by up-rooting plants and dispersing them, thereby enhancing opportunities for gene flow (Boström 1995). In other words, gene flow can act as a facilitating force rather than as a constraining force on selection. We predict that *Z. marina* has the potential to maintain itself along European coastlines so long as habitat fragmentation is minimized and coastal development carefully controlled to protect seagrass habitat.

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Supplementary material

The following material is available from:
http://www.blackwellpublishing.com/products/journals/suppmat/Mec/MEC2205/MEC2205sm.htm

Table S1 Summary of genetic variation for all populations of *Zostera marina* (Table 1) based on nine microsatellite loci. Localities printed in italics belong to the Core populations and are used in some calculations. (N) = sample size (after duplicate genets were removed); N_A = number of alleles; H_{exp} = unbiased expected heterozygosity (Nei 1978); H_{ob} = observed heterozygosity; f = inbreeding coefficient and F_{ST} = global variance component contributed by an indivdual locus. Significant values (bold) have been applied after sequential Bonferroni correction (Rice 1989) (*P < 0.05, **P < 0.01, ***P < 0.001). DNA = locus Does Not Amplify; ---- = missing data (not null alleles) or locus is monomorphic. See Materials and methods for details of locus nomenclature.

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All of the contributing authors are keenly interested in one or more aspects of the genetics, ecology and conservation of seagrass ecosystems around the world. This work is a testimony to cooperation in achieving our long-term goal of understanding the evolution of *Zostera marina* in the entire Northern Hemisphere.
