ORIGINAL ARTICLE

Drifting fronds and drifting alleles: range dynamics, local dispersal and habitat isolation shape the population structure of the estuarine seaweed *Fucus ceranoides*

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ABSTRACT

Aim The seaweed *Fucus ceranoides* is restricted to spatially discrete estuarine habitats and lacks planktonic dispersal phases; it is therefore expected to exhibit strong population differentiation. Its cold-temperate affinities and mtDNA variation imply that the northern part of the species' range, where *F. ceranoides* is now ubiquitous, was recently colonized after the onset of the last deglaciation, potentially resulting in areas with greater genetic homogeneity. Here we examine the population structure of *F. ceranoides* to test these predictions, emphasizing the contrasting genetic signatures of limited dispersal in refugial versus recently colonized regions.

Location North-eastern Atlantic estuaries from Portugal to Norway.

Methods A total of 504 individuals from 21 estuarine sites spanning the entire range of *F. ceranoides* were sampled and genotyped for nine microsatellite loci. Population structure was inferred from several genotypic and allele-frequency analyses. Geographical patterns of genetic diversity were used to reconstruct the historical biogeography of the species.

Results Genetic diversity and regional population differentiation showed a consistent decline with increasing latitude. Southernmost populations harboured most of the endemic variation, whereas the northern populations (> 55° N) were almost fixed for the same alleles across loci. In southern and central regions of its distribution, *F. ceranoides* showed striking population subdivision, with many of the sampled estuaries corresponding to coherent genetic units that were easily discriminated from one another with standard clustering methods.

Main conclusions The geographical pattern of genetic diversity supports the long-term refugial status of Iberia and a post-glacial range expansion of *F. ceranoides* into previously glaciated latitudes. Despite the species' capacity to colonize newly available habitats, the genetic structure of *F. ceranoides* outside the recently (re)colonized range reveals that gene flow between populations is extremely low. This study provides a remarkable example of how infrequent and spatially limited dispersal can have contrasting effects at the scales of meta-population (connectivity) versus range dynamics (habitat tracking), and of how dispersal restrictions can result in either genetic divergence or homogeneity depending on the maturity and demographic conditions of the populations.

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INTRODUCTION

Over the Quaternary period, the historical distribution of ecosystems and species has been mediated largely by the alternation of cold glacial and temperate interglacial conditions (Hofreiter & Stewart, 2009). During glacial peaks, such as the Last Glacial Maximum (LGM, *c.* 20 ka), temperate taxa typically persisted in southern refugial areas, whereas interglacials (such as the present one) allowed the (re)colonization of more northerly latitudes (Taberlet *et al.*, 1998; Hewitt, 1999; Petit *et al.*, 2002). This classical glacial contraction/interglacial expansion model is central to phylogeographical research, and helps explain the common poleward decrease of genetic diversity exhibited by terrestrial taxa (Hewitt, 1999; Hampe & Petit, 2005). Species responses to climatic oscillations have nevertheless taken more diverse and idiosyncratic forms (Stewart & Lister, 2001; Gómez & Lunt, 2007; Svenning *et al.*, 2008), depending on the specific ecological adaptations and life history traits displayed by individual taxa (Bennett & Provan, 2008; Bhagwat & Willis, 2008; Stewart & Dalén, 2008; Stewart *et al.*, 2010).

The effects of the Pleistocene glaciations on coastal ecosystems remain less well understood. Given the correlated latitudinal fluctuations of air and sea surface temperature (SST) isotherms, the terrestrial paradigm of 'southern refugia' has frequently been the null expectation when interpreting the range-wide patterns of genetic variability of coastal marine species. In the north-eastern Atlantic, several species do show clear genetic signatures of post-glacial expansions within previously glaciated latitudes (e.g. Hoarau *et al.*, 2007; Remerie *et al.*, 2009), but genetic patterns are variable among taxa (Wares & Cunningham, 2001; reviewed in Maggs *et al.*, 2008) and many do not show the expected negative correlation between latitude and diversity (Olsen *et al.*, 2004, 2010; Roman & Palumbi, 2004).

Strict analogies between terrestrial and marine environments may be inappropriate, because biological assemblages, geographical templates, environmental gradients and dispersal patterns are fundamentally different between the two realms (Graham *et al.*, 2003; Kinlan & Gaines, 2003; Lomolino *et al.*, 2005; Harley *et al.*, 2006). The inferred location of marine refugia, for instance, frequently differs from terrestrial settings. The ice-free palaeo-shorelines of Brittany, the English Channel and south-western Ireland have consistently been recognized as northern, periglacial refugia (in addition to more southerly refugia) for a range of invertebrate and seaweed species (Provan *et al.*, 2005; Gómez *et al.*, 2007; Hoarau *et al.*, 2007; Remerie *et al.*, 2009; Olsen *et al.*, 2010), despite the absence of most of their modern terrestrial counterparts. Moreover, instead of showing signatures of stable rear edges, southernmost populations of some coastal species are genetically impoverished (but see Provan & Maggs, 2012), presumably as a consequence of the progressive marginality of the habitat and continuing demographic instability brought about by the interglacial warming (Coyer *et al.*, 2003; Olsen *et al.*, 2010).

The molecular reconstruction of the historical biogeography of a species is inseparable from idiosyncrasies of ecology and life history, particularly habitat configuration and mode of dispersal. These are key factors constraining the scale of connectivity of populations, metapopulation regulation and spread (colonization) rates, all processes known to influence the patterns of genetic diversity and structure of species at multiple temporal and spatial scales (Ibrahim *et al.*, 1996; Irwin, 2002; Kinlan *et al.*, 2005; Cowen & Sponaugle, 2009). Marine organisms displaying low vagility and/or living in discontinuous seascapes are appropriate models for such molecular approaches. They are prone to accumulate (and retain) genetic differences and typically allow the examination of historical and contemporary patterns of vicariance, gene flow and colonization (Dawson *et al.*, 2001; Kelly *et al.*, 2006; Remerie *et al.*, 2009).

The cold-temperate, European endemic fucoid seaweed *Fucus ceranoides* L. (horned wrack) is perennial and dioecious. It is restricted to estuarine intertidal areas under fluctuating salinities, where it forms relatively small and isolated populations. As fucoids have no planktonic dispersal stages and gamete dispersal is very local (< 50 m; Chapman, 1995; Serrão *et al.*, 1997; Dudgeon *et al.*, 2001), its populations presumably persist mostly via self-recruitment. The modern distribution of *F. ceranoides* ranges from the River Mondego in Portugal (41° N) to northern Norway (70° N; Lein, 1984) and Iceland (Munda, 1999), encompassing both previously glaciated and non-glaciated regions of Europe.

A recent mtDNA survey showed that the southernmost *F. ceranoides* populations of north-western Iberia harbour two endemic, highly differentiated lineages, indicating a possible Pleistocene climatic refugium for the species (Neiva *et al.*, 2010; reproduced in Fig. 1). Outside Iberia, however, over large areas *F. ceranoides* exhibits an introgressed, *Fucus vesiculosus*-derived mtDNA lineage associated with the species' post-glacial, poleward range expansion (Neiva *et al.*, 2010). The extent of introgression and low polymorphism limited the phylogeographical resolution of the mtDNA marker. Because sequence and typing data show that organellar capture was not accompanied by nuclear introgression (Billard *et al.*, 2005a; Neiva *et al.*, 2010), here we examine, using nuclear microsatellite markers, the population genetic structure of *F. ceranoides* throughout its whole distributional range. Our aim is to reconstruct the historical biogeography of *F. ceranoides*, and to evaluate the effects of limited dispersal and recent range shifts on the geographical organization of its genetic variation. We were particularly interested in testing the prediction that *F. ceranoides* exhibits considerably more diversity and among-population differentiation in southern (refugial) than in northern (recently colonized) regions.

MATERIALS AND METHODS

Sampling, DNA extraction and genotyping

Fucus ceranoides populations were sampled during 2008 from 21 estuaries from Portugal to Norway, covering the entire current distribution of the species in the north-eastern Atlantic
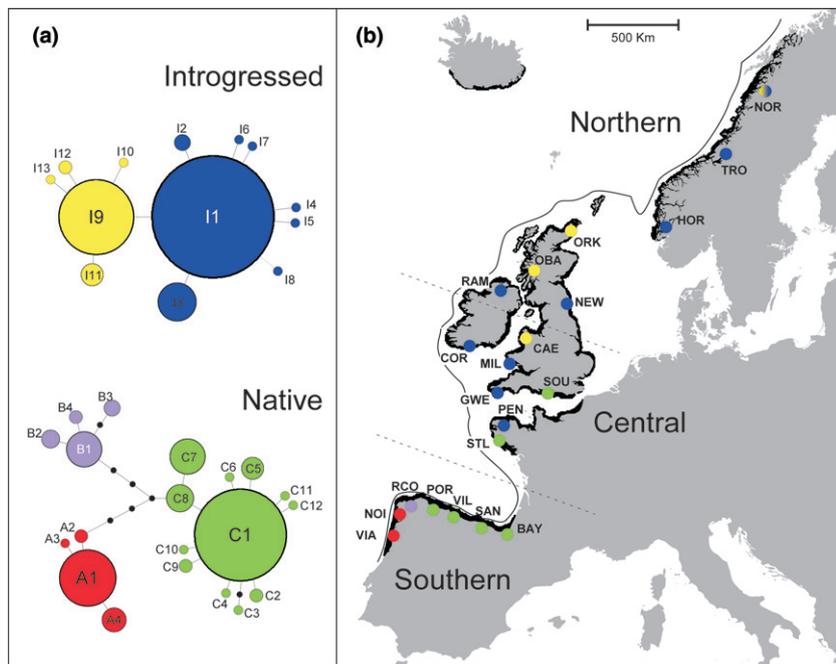
Figure 1 Distributional range of *Fucus ceranoides* in the north-eastern Atlantic, sampling sites and genetic subdivision inferred from a mitochondrial intergenic spacer, mtIGS (Neiva *et al.*, 2010). (a) Parsimony networks of native (lineages N_A, red; N_B, purple; N_C, green) and *F. vesiculosus*-derived (introgressed, lineage I, blue and yellow) mtIGS haplotypes. Sampled haplotypes are represented by circles sized to their frequencies; black dots represent inferred, unsampled haplotypes. Links represent a single nucleotide change. (b) Sampling locations of *F. ceranoides* and distribution of mtIGS lineages. The black contour depicts the distribution of *F. ceranoides* (only inside estuaries along those coastlines), the straight dashed lines delimit the geographical subdivisions considered (southern, central and northern regions of the species' range), and the circles represent the sampling localities coded as in Table 1 and coloured according to the mtIGS lineages present. The solid line represents the approximate Last Glacial Maximum shoreline. Adapted from Neiva *et al.* (2010).

(Fig. 1 and Table 1) with the exception of Iceland, where different *Fucus* species were found at the sites where *F. ceranoides* was expected. Minimum marine distances between adjacent populations ranged from 140 to *c.* 1200 km. At each site, 5–10 cm tips of apical vegetative tissue were excised from 22–24 individuals sampled along a 50–200 m linear or random walk. The protocols for DNA extraction are detailed in Neiva *et al.* (2010).

After testing in a small panel of *F. ceranoides* the cross-amplification, scoring and polymorphism of 25 microsatellite loci previously developed for other *Fucus* species (Engel *et al.*, 2003; Wallace *et al.*, 2004; Perrin *et al.*, 2007; Coyer *et al.*, 2009; J.A. Coyer *et al.*, unpublished), nine microsatellite loci were selected and amplified to generate multi-locus genotypes for all *F. ceranoides* individuals (see Table S1 in Appendix S1 in the Supporting Information for sources, primers and amplification details). Polymerase chain reactions (PCRs) were performed in 10 µL total volume containing 1× GoTaq Flexi buffer (Promega, Madison, WI, USA), 1.5–2.0 mM $MgCl_2$, 125 µM each dNTP, 0.2 µM of labelled (FAM, NED or HEX) forward primers, 0.5 µM of reverse primers, 1U GoTaq® Flexi DNA Polymerase (Promega), and 1 µL of 1:100 diluted DNA template. In all PCRs an initial denaturation step (94 °C, 5 min) was followed by *n* cycles of 94 °C for 30 s, a primer-specific annealing temperature (T_a) for 15 s and 72 °C for 30 s,

ending with a final extension step at 72 °C for 10 min (Table S1). Amplified fragments were separated using an ABI PRISM 3130xl (Applied Biosystems, CCMAR, Portugal) automated capillary sequencer. Alleles were manually scored in STRand (Toonen & Hughes, 2001) using the 350 ROX™ size standard (Applied Biosystems).

Data analysis

Summary statistics of genetic diversity within populations, including allele frequencies, mean allelic richness (A), Nei's gene diversity (H_E), observed heterozygosity (H_O) and inbreeding coefficients (F_{IS}), were calculated with GENETIX 4.05 (Belkhir *et al.*, 1996–2004). The same statistics were also computed for three selected regions, each encompassing a set of seven populations, broadly corresponding to the southern (Iberia), central (Brittany, English Channel, Celtic and Irish seas) and northern (> 55° N) parts of the *F. ceranoides* range.

Genetic structure was assessed using both population (allele-frequency-based) and individual (genotype-based) approaches. Pairwise F_{ST} (θ; Weir & Cockerham, 1984) was estimated with GENETIX 4.05 (Belkhir *et al.*, 1996–2004), and D (D_{est}; Jost, 2008) was estimated with SMOGD 1.25 (Crawford, 2010). Because H_E was very variable among populations (see Results), isolation by distance (IBD) was analysed with D_{est} instead of

Table 1 Genetic diversity and F_{IS} estimates in *Fucus ceranoides* at each sampling site and within southern, central and northern regions of the species' range (in bold), based on nine microsatellite loci. N, number of individuals genotyped in each population; A, mean number of alleles per locus; H_E, Nei's gene diversity; H_O, observed heterozygosity; F_{IS}, multi-locus inbreeding coefficient. The right column shows previously published data on the mitochondrial intergenic spacer (mtIGS) locus (Neiva *et al.*, 2010). Lineages and haplotypes are listed for each locality and region and coded as in Fig. 1. Absolute frequencies of haplotypes are in parentheses (if $N > 1$).

Region			Microsatellites					mtIGS		
Site, administrative region, country	Latitude, longitude	Code	N	A	H_E	H_O	F_{IS}	N	Clade	Haplotypes
Southern			**167**	**7.33**	**0.613**	**0.358**		**168**	**N**	
Viana do Castelo, Norte, PT	41°41′ N, 8°48′ W	VIA	23	2.56	0.165	0.168	−0.018	24	N_A	A1(21),A2(2),A3
Ria de Noia y Muros, W Galicia, ES	42°47′ N, 8°53′ W	NOI	24	3.00	0.315	0.308	0.024	24	N_A	A1(17),A4(7)
Ria de A Coruña, N Galicia ES	43°18′ N, 8°21′ W	RCO	24	3.78	0.323	0.300	0.073	24	N_B	B1(15),B2(4),B3(3),B4(2)
River Porcia, W Asturias, ES	43°33′ N, 6°52′ W	POR	24	3.44	0.425	0.454	−0.068	24	N_C	C1(20),C2(2),C3,C4
Ria de Villaviciosa, E Asturias ES	43°29′ N, 5°25′ W	VIL	24	2.78	0.408	0.426	−0.045	24	N_C	C1(17),C5(6),C6
Marismas de Santoña, Cantabria, ES	43°23′ N, 3°27′ W	SAN	24	3.22	0.401	0.407	−0.017	24	N_C	C7(15),C8(9)
Bayonne, S Aquitaine, FR	43°29′ N, 1°28′ W	BAY	24	3.44	0.463	0.431	0.071	24	N_C	C1(21),C9(2),C10
Central			**168**	**4.78**	**0.336**	**0.164**		**165**	**N, I**	
Anse de Saint Laurent, S Brittany, FR	47°54′ N, 3°56′ W	STL	24	3.00	0.316	0.306	0.033	24	N_C	C1(22),C11,C12
Penze, N Brittany, FR	48°36′ N, 3°56′ W	PEN	24	1.56	0.126	0.123	0.027	24	I	I1(21),I2(3)
Southampton, S England, UK	50°55′ N, 1°22′ W	SOU	24	1.22	0.022	0.023	−0.075	22	N_C	C1(22)
Gweek, SW England, UK	50°05′ N, 5°12′ W	GWE	24	1.67	0.091	0.111	−0.229*	24	I	I1(24)
Milford Haven, S Wales, UK	51°42′ N, 5°01′ W	MIL	24	1.89	0.092	0.088	0.037	24	I	I1(6),I3(18)
Caernarfon, N Wales, UK	53°08′ N, 4°16′ W	CAE	24	1.78	0.140	0.144	−0.030	24	I	I1,I9(22),I10
Cork, Cork, IE	51°54′ N, 8°28′ W	COR	24	2.78	0.339	0.352	−0.039	23	I	I1(21),I4,I5
Northern			**165**	**3.00**	**0.087**	**0.060**		**164**	**I**	
Ramelton, Donegal, IE	55°02′ N, 7°38′ W	RAM	24	2.00	0.091	0.097	−0.071	24	I	I1(22),I6,I7
Oban, W Scotland, UK	56°33′ N, 5°14′ W	OBA	22	1.89	0.133	0.141	−0.068	22	I	I1,I9(15),I11(6)
Orkneys, N Scotland, UK	58°58′ N, 03°15′ W	ORK	24	1.11	0.013	0.014	−0.045	22	I	I9(22)
Seaton Sluice, NE England, UK	55°04′ N, 01°28′ W	NEW	23	1.56	0.121	0.103	0.146	24	I	I1(23),I8
Hardangerfjord, Hordaland, NO	60°09′ N, 05°54′ E	HOR	24	1.22	0.052	0.060	−0.173	24	I	I1(24)
Tromdheimsfjord, Nord-Trondelag, NO	64°00′ N, 11°29′ E	TRO	24	1.33	0.014	0.014	0.007	24	I	I1(24)
Folda, Nordland, NO	67°21′ N, 15°35′ E	NOR	24	1.00	0	0	−	24	I	I1(12),I9(9),I12(2),I13

*$P < 0.05$ (1000 permutations). PT, Portugal; ES, Spain; FR, France; UK, United Kingdom; IE, Ireland; NO, Norway.

F_{ST}-based measures. IBD was evaluated for full and regional data sets using reduced major-axis regressions of pairwise estimates of D against minimum marine distances, as measured in Google Earth 5.1. The statistical significance of the genetic/geographical associations (1000 randomizations, $\alpha = 0.05$) was assessed with Mantel tests in IBDWS 3.15 (Jensen *et al.*, 2005).

In order to illustrate the degree of association between genotypic variation and geography, a neighbour-joining (NJ) network was generated from a matrix of pairwise Cavalli-Sforza & Edwards (1967) genetic distances of individuals (genotypes), using POPULATIONS 1.2 (Langella, 1999). Population genetic structure was further tested at the regional level (southern and central regions only) with a Bayesian, model-based genetic admixture analysis implemented by STRUCTURE 2.3 (Pritchard *et al.*, 2000; Falush *et al.*, 2003). Individuals were combined into one data set for analysis, without any *a priori* population assignments, and admixture was allowed. Each number of assumed populations (K, set sequentially from 1 to 9) was run five times using a burn-in of 100,000 iterations and a run-length of 500,000 iterations, which was determined to be sufficient to have consistent results. The 'true' value of K

was inferred both from the posterior probability of the data, hereafter referred to as $L(K)$, and following the ΔK choice criterion of Evanno *et al.* (2005), better suited to detecting heterogeneous patterns of dispersal or co-ancestry.

Recent migration rates among southern and central populations were estimated using a Bayesian MCMC analysis with BAYESASS 1.3 (Wilson & Rannala, 2003). Individuals were pre-assigned to populations based on sampling location. The analysis parameters were a burn-in of 1,000,000 iterations, a run-length of 3,000,000 iterations (sampling every 2000), and the default delta value of 0.15 for allele frequency, migration rate and level of inbreeding.

RESULTS

In total, 500 multi-locus genotypes of *F. ceranoides* from the 21 estuarine sites along the north-eastern Atlantic were produced using the nine selected microsatellite loci. Microsatellite polymorphism varied from 2 to 15 alleles per locus, with a mean (total allelic richness) of 8.56, dropping to 5.33 when only the alleles with a global frequency above 0.01 were considered. Most populations were monomorphic (or nearly fixed) for at least one

locus, frequently more, especially outside Iberia (Fig. S1 in Appendix S2). H_E ranged from 0 (the northernmost population, NOR, fixed for a single allele at all nine loci analysed) to 0.463 (BAY) (Table 1). H_O was not significantly different from H_E, with the exception of the GWE population, where heterozygote excess was detected at locus L58.

Both A and H_E estimators of genetic diversity showed a marked decline with latitude, at the population level but especially at the regional level (Fig. S2 in Appendix S2). On average, H_E was *c.* 6 times higher in the southern populations ($H_E = 0.357 \pm 0.100$) than in the northern populations ($H_E = 0.060 \pm 0.055$), and *c.* 7 times higher when compared for the regions as a whole (Fig. 2a). The central populations showed intermediate levels of genetic diversity but larger variation among sites ($H_E = 0.161 \pm 0.120$).

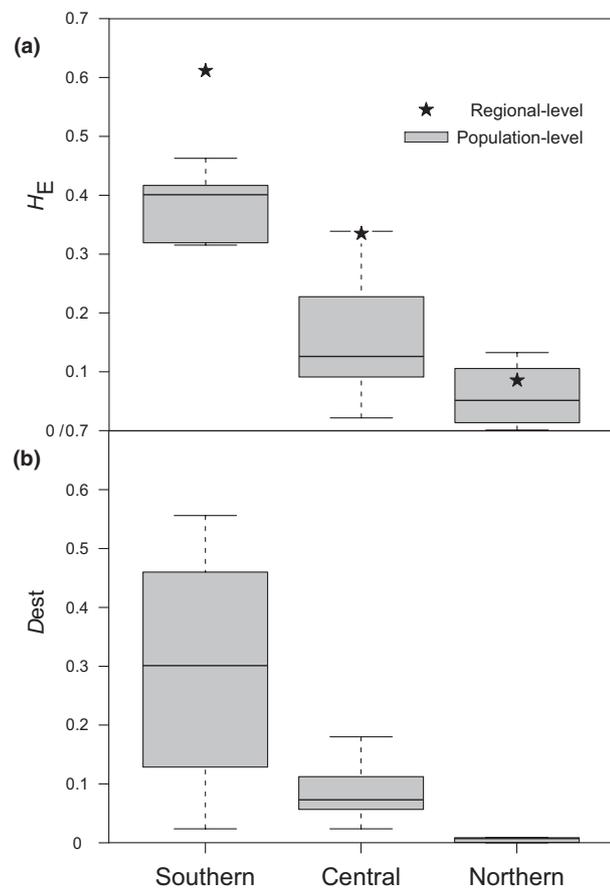All but three pairs of populations showed significant differentiation ($P < 0.05$), with strikingly extreme values of F_{ST} ranging from 0.007 (the two northernmost populations, essentially fixed for the same genotype) to 0.954 (SOU versus NOR, nearly fixed for different genotypes) (Table S2 in Appendix S1). Globally, 184 (87.6%) of the 210 pairwise F_{ST} estimates were greater than 0.3; 131 (62.4%) were greater than 0.5; and 51 (24.3%) were greater than 0.7. Absolute population differentiation, as measured by D_{est}, ranged from ≤ 0.001 (several pairwise comparisons among northern populations) to 0.582 (NOI versus STL; Table S2). Within regions, the highest D_{est} values were found in population pairs from the southern region, but this was also the region with more variable D_{est} (0.070–0.556; Fig. 2b). In contrast, D_{est} values were lower and more homogeneous within central Europe (0.024–0.180), and basically null (< 0.029) among northern populations.

No pattern of IBD was detected for the whole data set ($P = 0.090$), although a weak significant relationship ($P = 0.002$) was observed for the subset of 'Core' populations, after the six most peripheral populations were removed from the analysis, including the highly differentiated north-western
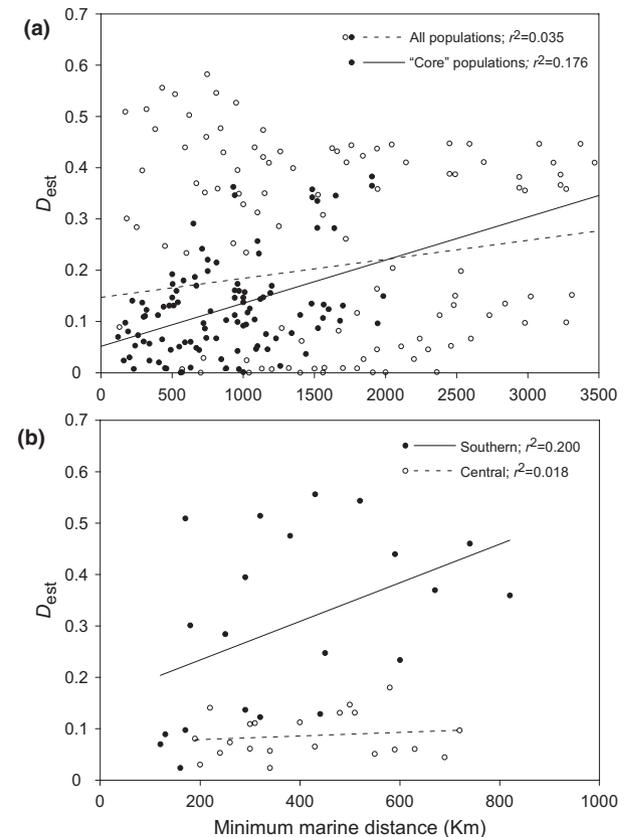

Figure 2 Genetic diversity and differentiation of populations of *Fucus ceranoides* within southern, central and northern regions of the species' range in the north-eastern Atlantic. (a) Nei's gene diversity (H_E) at population (box-plot, $N = 7$) and regional (stars) levels. (b) Box-plot of pairwise differentiation of populations (D_{est}) within regions ($N = 70$). Box-plots depict the median (horizontal line), the 25th and 75th percentiles (bottom and top of the box) and the minimum/maximum values (vertical dashed lines).

Figure 3 Isolation by distance in *Fucus ceranoides*. Estimates of pairwise differentiation (D_{est}) plotted against minimum marine distance in kilometres for (a) all populations (dashed line) and 'core' populations (solid line), and (b) populations from southern (dashed line) and central (solid line) regions of the species' range. The regressions are: $y = 3.704 \times 10^{-5}x + 0.147$ (all populations), $y = 8.410 \times 10^{-5}x + 0.051$ ('core' populations), $y = 3.752 \times 10^{-4}x + 0.159$ (southern region) and $y = 3.454 \times 10^{-5}x + 0.072$ (central region).

Iberian populations of VIA, NOI and RCO, as well as the nearly identical, geographically distant Norwegian populations (Fig. 3a). At the regional level, a nearly significant IBD relationship was observed within Iberia ($P = 0.053$), but not in the English Channel ($P = 0.309$; Fig. 3b). This analysis was not relevant for the northern region, given the very low level of differentiation between populations.

The NJ network (Fig. 4) highlighted the greater differentiation of several Iberian genotypic/geographical/mitochondrial clusters (e.g. VIA/NOI, RCO, POR/VIL) and the higher regional diversity within Iberia (southern region) compared with the central/northern cluster. The network also showed that *F. ceranoides* multi-locus genotypes were strongly clustered geographically. Throughout the southern and central parts of the distributional range there was a remarkable correspondence between genotype clusters and single (or occasionally two) estuarine sites. In the northern region the pattern was completely the opposite, with most individuals basically fixed across loci for the same alleles, thus forming a single cluster with no geographical resolution. All genotypes from Brittany northwards (i.e. all central and northern regions, excluding SOU) formed a rather cohesive cluster that was well differentiated from the southern genotypes. Within the latter, the southernmost Iberian genotypes (VIA/NOI and RCO) formed the clusters most differentiated from all others in the species.

Within southern and central regions, the STRUCTURE clustering analysis of the multi-locus genotypes also revealed
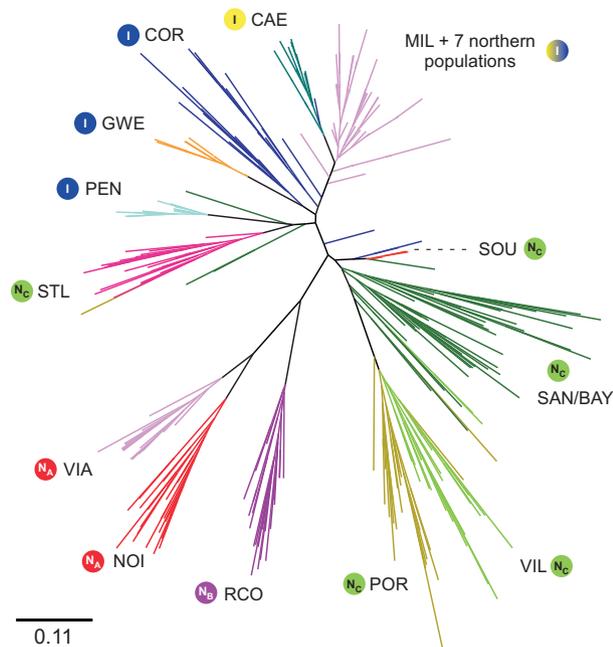
striking population subdivision down to the estuarine level (Fig. 5). *Fucus ceranoides* populations were hierarchically structured within Iberia (Fig. 5a). Based on the ΔK criterion of Evanno *et al.* (2005), the highest level of genetic/geographical subdivision occurred between VIA/NOI and the remaining populations of Iberia ($K = 2$), with VIA/NOI, RCO, POR/VIL and SAN/BAY ($K = 4$) representing a weaker level of population subdivision (Fig. S3a in Appendix S2). Alternatively, Pritchard *et al.*'s (2000) $L(K)$ criterion resolved six distinct genetic clusters matching specific estuarine sites (with the exception of VIA and NOI that clustered together), although suggesting an appreciable degree of admixture between SAN and BAY sites. In the central region, all seven estuarine sites sampled were largely resolved as distinct genetic clusters irrespective of the choice criterion used (Fig. S3b).

The Bayesian inference of migration rates indicates that, among populations within southern or central regions, recent immigration was generally lower than 0.02 individuals per generation (Table S3 in Appendix S1); that is, these populations are evolving rather independently. Nevertheless, estimated migration rates from SAN to BAY (southern region) and from MIL to CAE (central region), contiguous in both
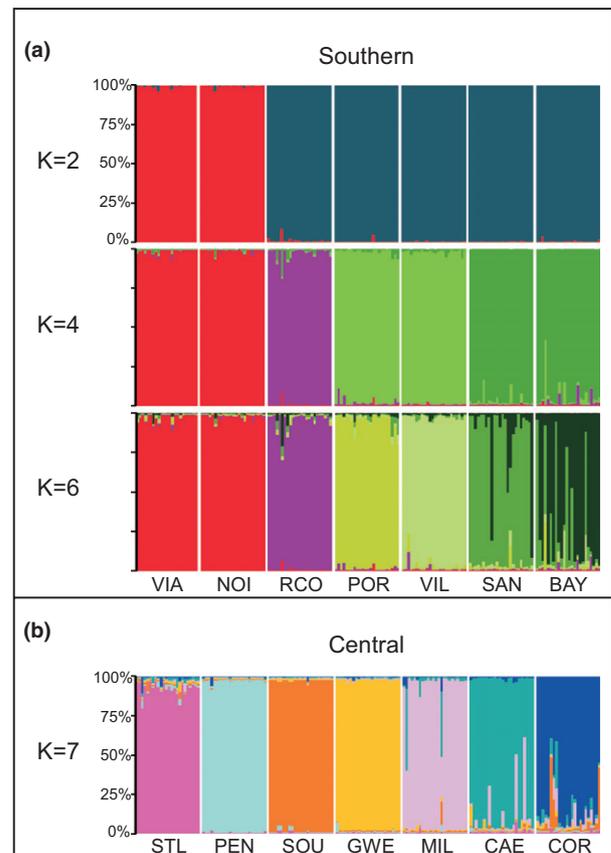

Figure 4 Neighbour-joining network of genotypes of *Fucus ceranoides* using Cavalli-Sforza & Edwards (1967) pairwise distances. The inferred genotypic/geographical clusters are shown in different colours and are accompanied by a circle representing the mitochondrial intergenic spacer (mtIGS) clades present. Population codes and mtIGS clade designations are given in Table 1.

Figure 5 Genetic subdivision of *Fucus ceranoides* in (a) southern, and (b) central regions of the species' range based on STRUCTURE. Shown are the proportions of individual multilocus genotypes assigned to each of the K virtual clusters, as illustrated by the different colours. Population codes are given in Table 1.

cases, were not negligible ($m = 0.292$ individuals generation^{-1}). These two pairs of populations do not, however, share the same mitochondrial intergenic spacer (mtIGS) haplotypes (23S ribosomal RNA/*trn*K intergenic spacer; Table 1), showing a lack of connectivity, at least for females, between these neighbouring populations.

DISCUSSION

Historical range dynamics

A clear poleward decrease in allelic richness, gene diversity and within-region population differentiation was revealed by the comparison of southern, central and northern populations of *Fucus ceranoides*. The southern region (i.e. Iberia) harbours many private alleles (in addition to two endemic mtIGS lineages), and populations there were grouped hierarchically in small shoreline sectors (< 200 km). These were strongly differentiated from each other and from more northerly samples. This pattern of 'southern richness' is characteristic of temperate terrestrial taxa that have persisted throughout past glacial conditions in southern refugia (Hampe & Petit, 2005); it therefore supports the hypothesis of the long-term persistence of *F. ceranoides* in north-western Iberia, and possibly in northern Iberia as a whole.

It remains unclear whether *F. ceranoides* persisted throughout the last glaciation in parts of the central, periglacial region. Although several intertidal seaweeds (Provan *et al.*, 2005; Hoarau *et al.*, 2007; Olsen *et al.*, 2010), estuarine gobids (Gysels *et al.*, 2004) and estuarine mysids (Remerie *et al.*, 2009) persisted there throughout the LGM, the particular inland (upper estuarine) and intertidal distribution of *F. ceranoides* implies that this species would have been more exposed to the harshness of the environment (e.g. winter freezing, ice scouring). In general, the genetic diversity in the central region is considerably lower than that in the southern region, and the allelic variants represent only a subset of the diversity present in Iberian populations. The few exceptions probably originated locally after colonization, rather than representing older variation specific to this area. The lower diversity and lack of endemism in the central region thus support a non-refugial status for this area, but the alternative hypothesis of a second refugial zone there cannot be ruled out for two reasons: (1) the central/northern genotypes form a rather distinct and cohesive cluster in the NJ network; and (2) central populations are genetically distinct from each other, much more so than those at northern latitudes that show an unambiguous signature of a recent colonization from a single source.

It is possible that the lower diversity in the central region is not the result of post-glacial colonization events, but rather of bottlenecks that have eroded pre-existing variation despite a continued presence in the area (Brochmann *et al.*, 2003; Bennett & Provan, 2008). Such bottlenecks could have resulted from limited estuarine availability (owing to the extensive permafrost belt covering continental Europe) and increased demographic instability caused by the marginal temperature regimes at this putative glacial trailing edge. The indirect effects of sea-level oscillations, which periodically transfigured the geography of the region, may have contributed as well. Significant shoreline displacement (up to several hundred kilometres) accompanying the regression/transgression of the shallow Celtic and Irish seas, and also of the English Channel (Gibbard & Lautridou, 2003; Ménot *et al.*, 2006), caused the regional migration of near-shore habitats, including estuaries. Physical shifts and displacement of habitat leading to local extinctions/recolonizations would introduce an additional source of bottlenecks, and have the potential to leave the genetic signature of a 'real' regional colonization.

The expected post-glacial range expansion of *F. ceranoides* into previously glaciated latitudes is strongly supported, as the extremely low microsatellite gene diversity and lack of population differentiation in the northern region represent good empirical evidence of a severe and recent colonization sweep (Pascual *et al.*, 2001; Excoffier *et al.*, 2009). Indeed, northern populations were extremely homogeneous, despite the distance between many samples exceeding 1000 km. Although the microsatellite data support an expansion originating from the central region, they fail to clearly delineate colonization pathways. Still, the distribution of the two most common haplotypes (I1 and I9, see Neiva *et al.*, 2010) suggests that colonization proceeded via two distinct phases and routes, as in the estuarine mysid *Neomysis integer* (Remerie *et al.*, 2009). The first wave migrated northwards following the submersion of the Celtic and Irish seas, during which time the originally introgressed haplotype I1 was replaced by the derived haplotype I9, which eventually spread further north along Scotland. After the re-establishment of the passage between the Channel and the North Sea (*c.* 7.5 ka), a second wave, also bearing the 'ancestral' haplotype I1, expanded along the previously emerged English Channel, then northwards along the east coast of England and ultimately reached the Norwegian fjords.

The 'southern richness–northern purity' genetic pattern of *F. ceranoides* contrasts with those found in other cold-temperate fucoids with which it shares the same general distribution in the north-eastern Atlantic. The highest levels of genetic diversity in *F. serratus* and *Ascophyllum nodosum* are found in Brittany, the English Channel and south-western Ireland, that is, in more central regions of their extant distributions, while populations at their common, southern distributional boundary are comparatively impoverished (Coyer *et al.*, 2003; Hoarau *et al.*, 2007; Olsen *et al.*, 2010). These species have more restricted horizontal and vertical distributions in Iberia (Pearson *et al.*, 2009; Araújo *et al.*, 2011), and also exhibit more unstable demographies and range dynamics there (Arrontes, 1993, 2002; Fernández & Anadón, 2008; Viejo *et al.*, 2010). The highest levels of genetic diversity and endemism for *F. ceranoides* observed in this same southernmost region show that the area still represents a stable rear edge that has not turned into a marginal habitat during the course of the present interglacial. Unique genetic

variation was also detected in Iberian populations of the red seaweed *Chondrus crispus* (Provan & Maggs, 2012), suggesting that this restricted and marginal region may represent an important repository of genetic diversity in several other intertidal organisms with similar geographic distributions.

Drifting fronds and drifting alleles

Along the southern distributional range, besides long-term persistence, long-term isolation is also revealed by the strikingly high differentiation between populations. The sharp discontinuities revealed by these new microsatellite data agree with a previous mtDNA survey (Neiva *et al.*, 2010), but provide more resolution of inter-estuarine differences. Where regional genetic diversity allowed a minimum resolution (southern and central regions), even the most contiguous estuaries contained much differentiated populations. Such a remarkable genetic structure, concordant for both nuclear and mitochondrial markers, thus validates the prediction of very restricted inter-estuarine gene flow in *F. ceranoides*.

Significant (but weak) IBD was seen only when the six most peripheral populations were removed from analysis. In the southern region, contiguous populations could be highly differentiated (e.g. Noi/Rco or Rco/Por pairs) or not (Por/Vil or San/Bay pairs), and in the central region differentiation between any pair of populations was of the same order of magnitude, irrespective of distance. Clearly, the distance between populations is not the single factor shaping modern patterns of differentiation at these scales (> 100 km). Lack of IBD has been reported for other seaweed and seagrass species with limited dispersal and/or fragmented distributions (e.g. Billot *et al.*, 2003; Arnaud-Haond *et al.*, 2007; Alberto *et al.*, 2010; Fraser *et al.*, 2010), including other *Fucus* species (Coyer *et al.*, 2003; Coleman & Brawley, 2005; Tatarenkov *et al.*, 2007), but in all such cases distance typically explains considerably more spatial genetic variability than observed in *F. ceranoides*.

Gamete dispersal is very local in fucoid algae, and thus *F. ceranoides* individuals will interact and reproduce mostly within the discrete, isolated patches of estuarine habitat they inhabit. Within populations, no significant departures from Hardy–Weinberg equilibrium were found, suggesting random gamete mixing at the scale of the sampling design (i.e. for non-neighbouring individuals along 50–200 m linear distances). Given the larger dispersal capacity of *Fucus* sperm (> 10 m) than of eggs (most settle within 0.5 m, Serrão *et al.*, 1997), such local mixing within *F. ceranoides* populations is probably mediated by sperm dispersal rather than by eggs. Once they have been released, however, even in the odd case of sperm transport beyond the estuary, it is doubtful that they could mediate inter-estuarine gene flow given their short longevity, rapid dilution and sensitivity to variations in salinity (Serrão *et al.*, 1996).

Inter-estuarine (long-distance) dispersal can still be mediated by drifting fertile fronds that escape local hydrographical circulation patterns and continue to spawn (before senescence) when deposited in new estuarine sites, as reported for other algae (Hoek, 1987; Norton, 1992; McKenzie & Bellgrove, 2008). Drift material of *F. ceranoides* is occasionally found at river mouths and on nearby beaches (João Neiva, personal observation), but is unlikely to return to a suitable upper estuary. Even then, at least one reproductive individual of each sex would need to be present in close contact and synchronously releasing gametes in order to produce *in situ* the zygotes that might mediate gene flow or eventually establish a new population. Such a rare event would have been the only possible mechanism for the post-glacial colonization of northern Europe.

Inter-estuarine gene flow requires contact between a single immigrant and an established population. The regional population genetic structure of *F. ceranoides* is characterized by fixed haplotypic/allelic differences between populations and lack of IBD, which suggests that historical and recurrent (drift) processes contributing to population differentiation are weakly counteracted by ongoing gene flow. Effective migration via rafting must thus be rare and relatively erratic over most spatial scales (see Thiel & Haye, 2006). The effects of isolation are probably reinforced by drift under small effective population sizes (N_e; see Coyer *et al.*, 2008; Turner *et al.*, 2002). Small N_E values are expected from the typically small size of estuarine patches, habitat instability over ecologically and geologically relevant scales (Attrill & Rundle, 2002) and large variance in the reproductive success of individuals (Vernet & Harper, 1980; Billard *et al.*, 2005b; Pearson & Serrão, 2006).

Rafting may contribute little to the genetic connectivity of populations of *F. ceranoides*, but has played an important role during this species' post-glacial colonization of northern Europe. It was also the primary dispersal mechanism assisting the extensive post-glacial expansions of other seaweeds with restricted propagule dispersal (e.g. Fraser *et al.*, 2009), and the modern spread of several invasive seaweed species (Kinlan & Gaines, 2003; Lyons & Scheibling, 2009). Rare dispersal, however, is expected to constrain the expansion rate and the dynamics of the colonization front(s). The extreme genetic homogeneity of northern *F. ceranoides* shows that a severely bottlenecked leading edge was formed during the northward expansion. Such a pattern is seen in terrestrial taxa where the descendants of rare long-distance colonizers spread ahead of the core populations (Hewitt, 2000). In the case of *F. ceranoides*, its low genetic connectivity and narrow shoreline habitat indicate that even a short-range stepping-stone expansion would have entailed severe genetic bottlenecks, leading to fast genetic erosion at the leading edge (Austerlitz *et al.*, 1997) and to an increased probability of genetic surfing (Excoffier & Ray, 2008; Neiva *et al.*, 2010).

CONCLUSIONS

The palaeoclimatic oscillations in the North Atlantic have clearly driven important biogeographical shifts in *F. ceranoides* and played a significant role in shaping its genetic make-up. Our study supports the status of Iberia as a long-term (multiple glacial/interglacial cycles) refugium, and confirms

the post-glacial range expansion of *F. ceranoides* into previously glaciated latitudes but not its cryptic persistence in periglacial areas during the last glaciation. NW Iberia, the area of greatest genetic diversity and conservation value, did not participate in the post-glacial poleward expansion.

Fucus ceranoides lies at the low end of the marine dispersal continuum. Episodic dispersal by rafting contributes little to connecting populations of *F. ceranoides*, but allows the successful colonization of unoccupied shores. *Fucus ceranoides* provides a remarkable example of how infrequent and spatially limited dispersal can have contrasting effects at the scales of meta-population (connectivity) versus range dynamics (habitat tracking), and how dispersal restrictions can result in either genetic divergence (refugial areas) or homogeneity (recently colonized areas) depending on the maturity and demographic conditions of the populations.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Primer sequences, polymerase chain reaction conditions and sources of microsatellite loci (Table S1); pairwise differentiation between the 21 populations of *Fucus ceranoides* (Table S2); and migration rates among southern and central populations of *F. ceranoides* (Table S3).

Appendix S2 Microsatellite allele frequencies in each population of *Fucus ceranoides* (Fig. S1); latitudinal trends of genetic diversity in *F. ceranoides* (Fig. S2); and genetic subdivision of *F. ceranoides* into southern and central regions according to STRUCTURE (Fig. S3).

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