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Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*

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Abstract

The last glacial maximum (20 000–18 000 years ago) dramatically affected extant distributions of virtually all northern European biota. Locations of refugia and postglacial recolonization pathways were examined in *Fucus serratus* (Heterokontophyta; Fucaceae) using a highly variable intergenic spacer developed from the complete mitochondrial genome of *Fucus vesiculosus*. Over 1500 samples from the entire range of *F. serratus* were analysed using fluorescent single strand conformation polymorphism. A total of 28 mtDNA haplotypes was identified and sequenced. Three refugia were recognized based on high haplotype diversities and the presence of endemic haplotypes: southwest Ireland, the northern Brittany-Hurd Deep area of the English Channel, and the northwest Iberian Peninsula. The Irish refugium was the source for a recolonization sweep involving a single haplotype via northern Scotland and throughout Scandinavia, whereas recolonization from the Brittany-Hurd Deep refugium was more limited, probably because of unsuitable soft-bottom habitat in the Bay of Biscay and along the Belgian and Dutch coasts. The Iberian populations reflect a remnant refugium at the present–day southern boundary of the species range. A generalized skyline plot suggested exponential population expansion beginning in the mid-Pleistocene with maximal growth during the Eems interglacial 128 000–67 000 years ago, implying that the last glacial maximum mainly shaped population distributions rather than demography.

Keywords: brown algae, *Fucus serratus*, glacial refugia, mitochondria, phylogeography, seaweed

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Introduction

The effects of the Quaternary glacial-interglacial periods, especially the last glacial maximum (LGM, 18 000–20 000 years ago), on phylogeography of modern-day European flora and fauna have been a major research topic for more than a decade (Hewitt 1996; Hewitt 2000; Hewitt 2001). Advancing glacial ice forced species and populations into refugial areas from which they expanded when the ice receded. It is expected that the reduced geographical ranges associated with isolation into refugia will result in high genetic diversity within refugial areas and high genetic dissimilarity between refugia (Hewitt 1996; Hewitt 2004). The contemporary signature of this process results in Hewitt's so-called 'southern richness, northern purity'. Complicating the model, however, is the possibility that a candidate refugial area of high allelic or haplotypic diversity is actually the result of secondary contact, or an admixture of formerly discrete geographical populations and therefore, may not be a refugium. Conversely, a very recent colonization sweep and/or radically changed set of environmental conditions, such as those found in edge zones of the species range, could mask a former refugium resulting in present-day low diversity of a formerly high-diversity area. Reconstructing phylogeographical histories thus provides a dynamic description of species distributional histories against the backdrop of earlier climate change.

Patterns of population contraction and expansion have been analysed for many terrestrial and a few freshwater species (Consuegra *et al*. 2002; e.g. Kotlik & Berrebi 2001; Seddon *et al*. 2001), leading to widespread recognition of three main European terrestrial refugia on the Iberian, Italian, and Balkan Peninsulas. For marine species, a growing body of recent studies on northeastern Atlantic taxa suggest a Pleistocene refugium around the Iberian Peninsula

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for marine macroalgae (Stam *et al*. 2000; Coyer *et al*. 2003), seagrasses (Coyer *et al*. 2004a), bivalves (Luttikhuizen *et al*. 2003), rays (Chevolot *et al*. 2006) and teleosts (Gysels *et al*. 2004); the English Channel/Brittany area for macroalgae and seagrasses (Stam *et al*. 2000; Coyer *et al*. 2003; Olsen *et al*. 2004; Provan *et al*. 2005); and the southwestern coast of Ireland for macroalgae (Provan *et al*. 2005) and polychaetes (Jolly *et al*. 2006).

The rockweed *Fucus serratus* (Heterokontophyta; Fucaceae) is one of several fucoid species collectively viewed as bioengineering species and dominating the intertidal biomass of northern European shorelines (Seed & O'Connor 1981; Lüning 1990). The perennial, dioecious and sexually reproducing species naturally extends from the northern Iberian Peninsula to the White Sea and throughout the Skagerrak-Kattegat-lower Baltic Seas. Its presence in Iceland, the Faeroes, and Nova Scotia, however, is due to human introductions based on early phycological surveys and genetic analyses (Hay & MacKay 1887; Robinson 1903; Edelstein *et al*. 1971, 73; Coyer *et al*. 2006b). *Fucus serratus* is an excellent species for investigating glacial refugia and pathways of postglacial recolonization because its current distribution includes areas thought to be glacial refugia, as well as new areas exposed as the last ice sheet receded. Furthermore, gene flow appears to be restricted (microsatellite-based panmictic unit < 2 km, Coyer *et al*. 2003), which makes it potentially easier to follow the genetic signature of post-LGM recolonization as it is less likely to be erased by contemporary gene flow. In contrast, the genetic signature of historical events for high gene flow species has often been confused (thornback ray, Chevolot *et al*. 2006) and/or erased (European plaice, Hoarau *et al*. 2004). *Fucus serratus* thus provides an ideal model to study the effect of the LGM on low dispersal rocky intertidal species in the Eastern Atlantic.

In a previous study using seven microsatellite loci, *F. serratus* populations in the Brittany/western English Channel area were characterized by the highest levels of genetic diversity, but it was unclear whether the diversity was due to a putative glacial refugium or from an admixture of different recolonization events (Coyer *et al*. 2003). As sampling from Ireland, Scotland and Scandinavia was limited, the possibility of a cryptic refugium in these areas was not testable. Although similarly inconclusive, the low diversity found in northwestern Spain, suggested a bottleneck and unsuitable present-day environmental conditions at the southern boundary rather than a refugium.

In the present study, we analysed samples of *F. serratus* from its entire North Atlantic distribution using a newly developed and highly variable mitochondrial marker (Coyer *et al*. 2006a) that we developed from a comparative mitochondrial genome sequencing project (Oudot-Le Secq *et al*. 2001; Oudot-Le Secq *et al*. 2002; Oudot-Le Secq *et al*. 2006). The goals of the present study were to: (i) analyse phylogeographical structure of *F. serratus* across its entire range; (ii) identify true refugia and subsequent recolonization routes; and (iii) assess the degree to which the LGM affected the historical demography in *F. serratus*.

Materials and methods

Sampling and DNA extraction

Samples were collected from 33 locations covering the distributional range of *Fucus serratus* (Fig. 1, Table 1). At all locations, 26–50 individuals were collected at *c*. 1-m intervals along a transect line. For all individuals collected, a 1-cm piece was excised from four to six apical tips and placed into silica crystals for rapid dehydration and storage as described earlier (Coyer *et al*. 2002b), DNA was isolated from *c*. 40 mg of silica-dried tissue as previously described (Coyer *et al*. 2002b).

Polymerase chain reaction-single strand conformation polymorphism and sequencing

A smaller (*c*. 400-bp) fragment of the larger (600–700-bp) mitochondrial DNA (mtDNA) intergenic spacer used in Coyer *et al*. (2006a) was amplified by polymerase chain reaction (PCR) using the primers: FsmtL-F (5′-TCAATTGATTATTXTTGAAAAG-3′) which was 5′-fluorescently labelled with 6-FAM and FsmtL-R (5′-TCTCTTATAATAAAGGTAAATTCTT-3′) which was 3′-fluorescently labelled with HEX. All PCR amplifications consisted of 10-μL reaction volumes containing 1 μL of DNA, 1× reaction buffer (Promega), 0.2 mM of each dNTP, 2 mM $MgCl_2$, 0.5 U *Taq* DNA polymerase (Promega) and μM of each primer. PCR was performed in either a PTC-100 (MJ Research) or a MasterCycler gradient cycler (Eppendorf). The reaction profile was: 90 °C for 1 min; 30 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min.

Single strand conformation polymorphism (SSCP) (Orita *et al*. 1989) was used to detect sequence variation in the mtDNA intergenic spacer. Mutations that affect the conformation of single-strand DNA are revealed by migration in nondenaturing polyacryalamide gels. This technique is very sensitive and provides a quick and relatively inexpensive way to screen a large number of samples for sequence variation. SSCP gels were run on an ABI PRISM 377 (Applied Biosystems) as described in Coyer *et al*. (2002a) except that 5% glycerol was added to the gels in order to enhance resolution. Because mutations can affect the mobility of one or both strands differently, separate labelling of each DNA strand increases the sensitivity and reliability of the SSCP.

SSCP gels were analysed independently and all differing haplotypes were subsequently sequenced. For each gel, the most common haplotypes were sequenced for at least

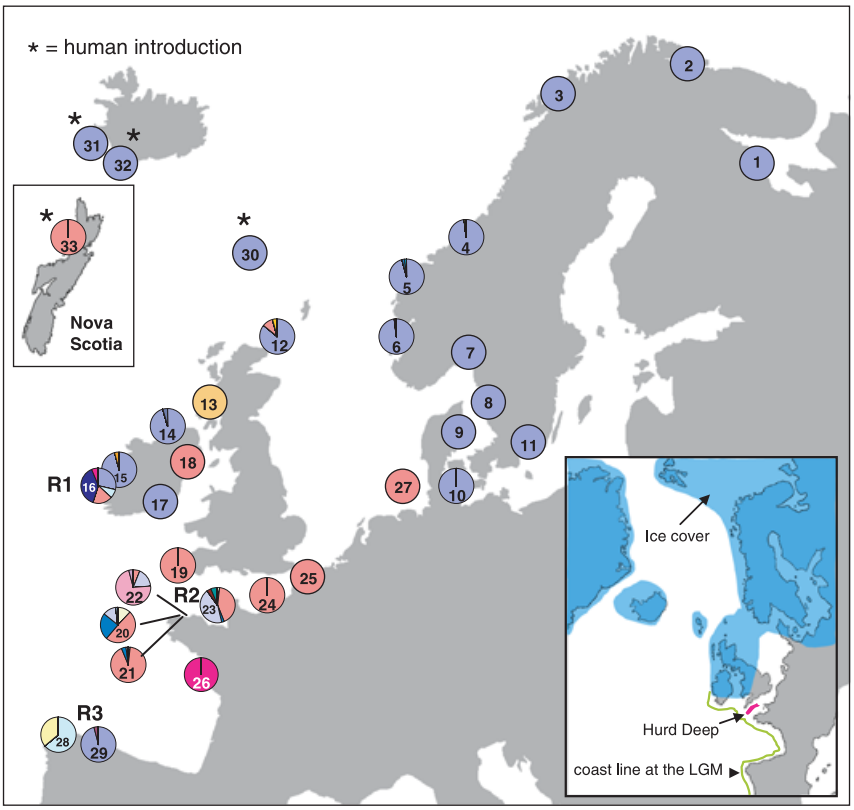

Fig. 1 Distribution of *Fucus serratus* mtDNA haplotypes. Numbers are the location IDs (see Table 1). Potential refugia are indicated by 'R'. Insert depicts putative ice cover and coastline at the last glacial maximum.

two individuals. PCR products were cleaned using either SigmaSpin (Sigma) purification columns or ExoSapIt (USB Corporation) enzyme following the provider's instructions. Both strands were cycle-sequenced using the dGTP Big Dye Terminator Kit (Applied Biosystems) and visualized on an ABI PRISM 377 (Applied Biosystems). Sequences were edited and aligned manually using BIOEDIT version 7.0.1 (Hall 1999). Identical SSCP profiles always yielded identical sequences.

Data analysis

Haplotype (*h*) (Nei 1987) and nucleotide (π) (Nei 1987) diversities were estimated using ARLEQUIN version 3 (Excoffier *et al*. 2005). Intraspecific relationships among the mtDNA haplotypes were inferred using statistical parsimony with the software TCS version 1.13 (Clement *et al*. 2000) with indels coded according to Barriel (1994).

Historical demographic changes were inferred using a generalized skyline plot (Strimmer & Pybus 2001). The method derives from coalescence theory (Kingman 1982), as the shape of a genealogy depends on the demographic history. More specifically, the generalized skyline plot uses a step function and the genealogy to represent estimated changes in effective population size (N_e) over time. It is important to keep in mind that the method provides a trend rather than a detailed estimate, assumes clock-like evolution and does not account for phylogenetic error. As a first step, a maximum-likelihood (ML) ultrametric tree (equal root-to-tip distance for all lineages) was constructed with the HKY85 + G model of molecular evolution (Coyer *et al*. 2006a) and a molecular-clock assumption in PAUP version 4.0b10 (Swofford 2002) as clock-like branch lengths are required. Next, a generalized skyline plot was generated from the ML tree using GENIE 3.0 (Pybus & Rambaut 2002) with a smoothing algorithm to reduce the noise in the data while simultaneously preserving the demographic signal. The smoothing parameter (ε) was estimated using the 'maximize optimization' option.

The use of a universal mtDNA molecular clock is not possible in *Fucus* because of the great phylogenetic distance between heterokont algae and animals, plants, red algae, and green algae (Keeling *et al*. 2005). In addition, a direct calibration of the molecular clock for the Fucales is not possible because of the lack of a fossil record. Therefore, a clock for the *Fucus* mtDNA spacer was estimated indirectly as follows. The closest heterokont relatives of *Fucus* that have both a fossil record and an extensive sequence database are the diatoms (see Keeling *et al*. 2005). Diatoms have generation times equivalent to the fucoids, that is on the order of years to even a decade (L. Medlin, personal communication) so that generation time effects are irrelevant. We first estimated a clock for the photosystem II chloroplast protein D1 (*psb*A) in diatoms using the

Table 1 *Fucus serratus*. Sampling location and regional code (for Fig. 2), country and map identification number (for Fig. 1), number of individuals per sampling location (N), number of mtDNA haplotypes (N_h), haplotypes present (H_{id}), haplotype (h) and nucleotide (π) diversities, * indicates introduced populations

Location	Country	Map ID	N	N_h	H_{id}	h	π
White Sea (WS)							
Solovetski	RU	1	26	1	h1	0.0000	0.0000
Norway (Nw)							
Kirkenes	NO	2	49	1	h1	0.0000	0.0000
Tromsø	NO	3	50	1	h1	0.0000	0.0000
Trondheim	NO	4	50	2	h1, h7	0.0400	0.0001
Ålesund	NO	5	48	3	h1, h8, h9	0.0824	0.0002
Bergen	NO	6	50	2	h1, h11	0.0400	0.0045
Smaskjaer	NO	7	50	1	h1	0.0000	0.0000
Kattegat (K)							
Tjärnö	SE	8	50	1	h1	0.0000	0.0000
Blushøj	DK	9	48	1	h1	0.0000	0.0000
Kiel	DE	10	49	2	h1, h6	0.0408	0.0002
Baltic (Ba)							
Karlshamn	SE	11	49	1	h1	0.0000	0.0000
Scotland (Sc)							
Orkneys	GB	12	49	3	h1, h5, h12	0.2585	0.0478
Oban	GB	13	35	1	h21	0.0000	0.0000
Ireland (Ir)							
Giants Causeway	GB	14	44	2	h1, h3	0.0888	0.0002
Galway	IE	15	48	2	h1, h4	0.0816	0.0002
Limerick	IE	16	49	5	h1, h2, h10, h12, h27	0.7389	0.0916
Wexford	IE	17	50	1	h1	0.0000	0.0000
Bangor	GB	18	49	1	h12	0.0000	0.0000
Cornwall (C)							
Plymouth	GB	19	44	2	h12, h13	0.0455	0.0001
Brittany (Br)							
Pl. du Lividie	FR	20	49	5	h12, h15, h16, h19, h23	0.6837	0.0578
Pl. du Siec	FR	21	49	4	h12, h16, h19, h23	0.1573	0.0108
Roscoff	FR	22	50	4	h12, h17, h19, h22	0.4531	0.0216
Jersey	JE	23	50	6	h12, h13, h18, h19, h20, h23	0.6367	0.0120
Normandy (Nd)							
St Valérie en Caux	FR	24	50	2	h12, h14	0.0400	0.0001
Cap Gris-Nez	FR	25	50	1	h12	0.0000	0.0000
Vendée (V)							
Ile D'Yeu	FR	26	29	2	h16, h24	0.0690	0.0188
North Sea (NS)							
Helgoland	DE	27	50	1	h12	0.0000	0.0000
Spain (Sp)							
La Coruña	ES	28	50	2	h27, h28	0.4702	0.0156
Ribadeo	ES	29	50	3	h1, h25, h26	0.0792	0.0005
Faeroes* (F)							
Sudurøy	FO	30	50	1	h1	0.0000	0.0000
Iceland* (Ic)							
Sandgerði	IS	31	47	1	h1	0.0000	0.0000
Heimaey	IS	32	43	1	h1	0.0000	0.0000
Nova Scotia* (Nv)							
Inverness	CA	33	35	2	h4, h12	0.0571	0.0146

sequence divergence (7.76%) between *Haslea ostrearia* (Y15074) and *Skeletonema costatum* (Y15137) and a divergence time of 65–87 million years (Myr) (Medlin *et al*. 1996; Sorhannus & Fox 1999). Our *psb*A diatom clock is therefore 0.08% to 0.12%/Myr (7.76%/87 Myr and 7.76/65, respectively, with conservative rounding). We then used this *psb*A diatom clock to estimate the divergence time of two fucoids *Ascophyllum nodosum* and *Fucus vesiculosus* for which *psb*A sequences were available. Given the sequence divergence (1.28%) for *psb*A between the fucoids *A. nodosum*

(AY528844) and *F. vesiculosus* (DQ307679), their divergence time was estimated to be 10–16 Myr (1.28/0.08 and 1.28/0.12, respectively). With an mtDNA spacer sequence divergence of 32% to 34% between *A. nodosum* and *F. vesiculosus* (G. Hoarau, unpublished data) and the 10–16-Myr divergence time, we inferred a Fucaceae mtDNA spacer clock of 2% to 3.4%/Myr (32/16 and 34/10, respectively).

The issue of heteroplasmy

Mitochondrial DNA is still the main marker used in phylogeographical studies because of its predominantly uniparental inheritance and supposed lack of recombination, although these assumptions have been recently challenged in a wide range of organisms (Tsaousis *et al*. 2005; Xu 2005). The presence of more than one mitochondrial genotype in an individual or heteroplasmy, has been detected in animal, angiosperm and heterokonts (Coyer *et al*. 2004b; Kmiec *et al*. 2006). Heteroplasmy can lead to mtDNA recombination (Hoarau *et al*. 2002), which will affect the accuracy of phylogenetic reconstructions (Posada & Crandall 2002), inferences related to demographic history, and the application of molecular clocks (Schierup & Hein 2000). Geographically specific heteroplasmy has been detected in Baltic Sea populations of *F. serratus* for the *nad* 11 mitochondrial gene (Coyer *et al*. 2004b). The separate labelling of each DNA strand for SSCP allows an easy identification of heteroplasmic individuals. In the present analysis, there was no evidence of heteroplasmy for the mtDNA spacer as all 1539 samples showed SSCP patterns characteristic of single sequences (one single peak for the forward sequences and one for the reverse) (data available on request). Consequently, we feel confident that our phylogeographical analysis has not been compromised by heteroplasmy.

Results

Haplotype distribution

A total of 28 mtDNA haplotypes were recovered (h1–h28 with corresponding GenBank Accession nos EF547157–EF547184) from 1539 individuals, with 10 (30%) being unique. The most common haplotype (h1) was present in 58% of the samples, whereas the second most common (h12) was found in 25% of the samples. Both haplotypes displayed nearly disjunct distributions. Haplotype h1 was the most widespread, ranging continuously from southern Ireland to Scandinavia, where it was either found alone or with closely related haplotypes (Cluster 1) (Fig. 2). It was also found in one Spanish location. In contrast, haplotype h12 and closely related haplotypes (Cluster 2) were found from Vendée (south of Brittany) to the Irish and North Seas. Cluster 1 and 2 haplotypes were found jointly only in Ireland, Scotland and Nova Scotia. Five distinct haplotypes were identified in the Spanish populations, one from Cluster 1 (h1), and all four from Cluster 3. Of the four Cluster 3-haplotypes, three were endemic to Spain (h25, h26, h28) and one (h27) was also found in southwest Ireland. Populations of *F. serratus* from Iceland, The Faroes and Nova Scotia are human introductions. Only h1 was found in Iceland and The Faroes, whereas h12 and h4 were present in Nova Scotia.

Genetic diversity

Overall mtDNA diversity (h, π) was high, ranging from 0 to 0.7389 and from 0 to 0.0916, respectively (Table 1). Highest diversity was recorded in southwest Ireland (0.7389, 0.0916) followed by Brittany (0.1573–0.6837, 0.0108–0.0578) and Spain (0.0792–0.4702, 0.005–0.0156). Diversity was very low from the Baltic to the White Seas (0–0.0824, 0–0.0045).

Historical demography

The haplotype network for Cluster 1 exhibited a starlike topography, with all haplotypes only one step from the central h1. In contrast, networks for Clusters 2 and 3 revealed a slightly deeper starlike topology, implying somewhat older relationships. The minimum sequence divergences between Clusters 1 and 2 (0.455%), 1 and 3 (0.455%), and 2 and 3 (0.452%), all corresponded to a minimum divergence time of 230 000–130 000 years (with a clock of 2% to 3.4%/Myr), clearly indicating a divergence predating the LGM with shared ancestral polymorphisms accounting for the common haplotypes between Ireland and Spain and between Ireland and Brittany. The generalized skyline plot (Fig. 4) indicated exponential demographic growth from the mid-Pleistocene with a dramatic acceleration (1–2 orders of magnitude) *c*. 100 000 years BP (after the Warthe/Saale glaciation in Northern Europe). The periods of population growth correlate well with interglacial periods.

Discussion

A previous microsatellite-based study of *Fucus serratus* revealed the highest diversity in the Brittany area, suggesting that the area was a glacial refugium (Coyer *et al*. 2003). In the present study, the combination of a new mtDNA marker and an expanded sampling (now covering the entire distribution), have revealed cryptic refugia and putative recolonization pathways. Based on the topology of the haplotype network and the pattern of genetic diversity, we infer three glacial refugia: the southwest coast of Ireland (R1), the Brittany/Hurd Deep region (R2) and northern Iberian Peninsula (R3) (Fig. 1).

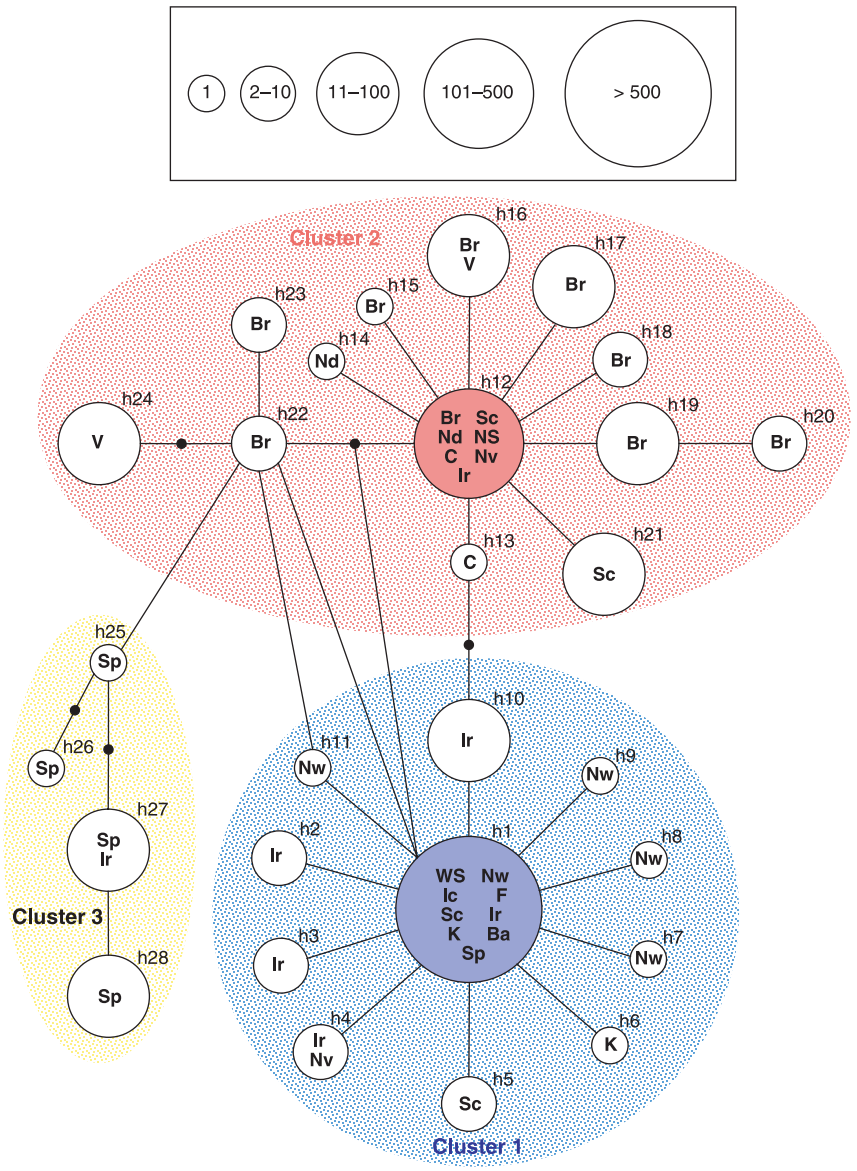

Fig. 2 Statistical parsimony network for 28 mtDNA haplotypes of *Fucus serratus*. Each circle represents a haplotype, with letters indicating the regions where each haplotype was found (Table 1). The size of the circle is proportional to the number of sampled individuals with a given haplotype. The h_x indicates haplotype number. Each line between haplotypes represents one mutational step. Missing haplotypes, indicated by small black circles with no identification, were either not sampled or extinct.

Southwestern Ireland refugium (R1)

The idea of a southwestern Irish glacial refugium stems from the biogeography of fauna and flora with a Lusitanian distribution (species co-occurring in southwest Ireland and Iberia but absent or with highly disjunct distributions in intermediate areas) (Praeger 1939; Vincent 1990). Independently, palaeoclimatic reconstructions of the LGM (Frenzel *et al*. 1992; Richter *et al*. 2001) provide direct evidence for an ice-free area. Recent phylogeographical studies of Scots pine (Sinclair *et al*. 1998) and natterjack toads (Rowe *et al*. 2006) suggested a terrestrial glacial refugium in southwestern Ireland. Moreover, evidence for a marine refugium in the coastal area has been found for the rocky intertidal red seaweed *Palmaria palmata* (Provan *et al*. 2005) and the soft-bottom polychaete worms *Pectinaria koreni* and *Owenia fusiformis* (Jolly *et al*. 2006). Our finding of highest genetic diversity and endemic haplotypes for *F. serratus* adds a fourth species to the marine list.

Brittany/English Channel refugium (R2)

During the LGM, the sea-level was *c*. 125 m below present levels and the nascent English Channel existed as a ice-free terrestrial depression (Frenzel *et al*. 1992) dissected by a canyon *c*. 150-km long and *c*. 100-m deep called the Hurd Deep (Fig. 1 inset) (Smith 1985; Lericolais *et al*. 2003). The Hurd Deep is thought to have persisted as a marine lake during the LGM until sea levels began to rise 15 000–13 000 years BP (sea level dates from Fleming *et al*. 1998)

and may well have been a suitable habitat for hard-substrate seaweeds as it is characterized by bedrock walls. Moreover, temperatures during the LGM were within the tolerance limits of many species including *Fucus* (Lüning 1984; Lüning & tom Dieck 1990; Arrontes 1993; Sarnthein 2001) and salinity was favourable as the major rivers of northern France turned westward and did not join the lake (Antoine *et al*. 2003). High microsatellite allelic diversity in the northern Brittany and Cornwall areas was first found in *A. nodosum* (Stam *et al*. 2000) and *F. serratus* (Coyer *et al*. 2003) but was not initially associated with the Hurd Deep hypothesis until Provan *et al*. (2005) correlated haplotype diversities from the red alga *P. palmata* and various palaeoclimatic data. The alternative hypothesis that the high allelic and haplotypic diversities characteristic of the Brittany/English Channel area are the results of admixture can be rejected, as all of the haplotypes detected in the area belong to cluster 2 (Fig. 2). The high population structure characteristic of *F. serratus* may account for the long-term maintenance of high levels of genetic diversity in such a small region.

The Iberian refugium (R3)

Signatures for a putative refugium (high diversity, private haplotypes) were also detected for the northwestern Iberian Peninsula. Present-day Iberian populations are at the southern distributional limit of *F. serratus* which is governed by temperature to the south and lack of suitable rocky substrate to the north. Sea-surface temperatures (annual mean) around the northwestern Iberian Peninsula increased from *c.* 10 °C 12 000 years ago BP to 18–19 °C 11 500 years ago BP (Bard *et al*. 2 000), rapidly switching the area from a glacial refugium to a marginal habitat for *F. serratus*. Thus, Iberian populations are now confined between a thermal barrier to the south and the Bay of Biscay to the north, which forms a second thermal barrier and unsuitable habitat for *F. serratus* (Arrontes 1993). During the past century, the distribution of *F. serratus* has expanded and contracted many times along the northern (west–east) Spanish coast (Arrontes 1993; Arrontes 2002) and massive mortality has been observed during extremely hot summers (Arrontes 1993). As repeated extinctions and recolonizations are evident in the reduced diversity together with very strong local differentiation based on microsatellite data (Coyer *et al*. 2003), we conclude that the Iberian populations are unstable remnants of a LGM refugium.

Recolonization pathways

The combination of extensive sampling and the restricted dispersal of *F. serratus* have allowed us to formulate a putative recolonization scenario for *F. serratus* along the Northeast Atlantic coast (Fig. 3). The recolonization sweep

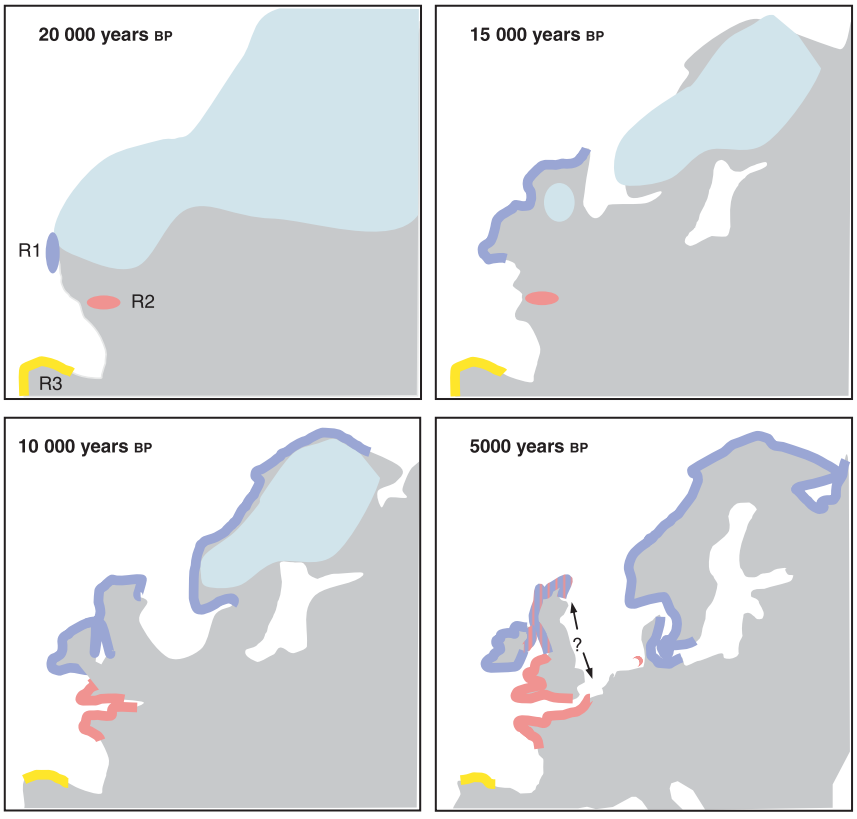

Fig. 3 Hypothetical phylogeographical history of *Fucus serratus* in Europe. R indicates refugia; colours indicate putative recolonization from the Irish refugium (R1) (blue); the Hurd Deep (R2) (pink); and the Iberian refugia (R3) (yellow). Insufficient sampling is indicated by '?'.

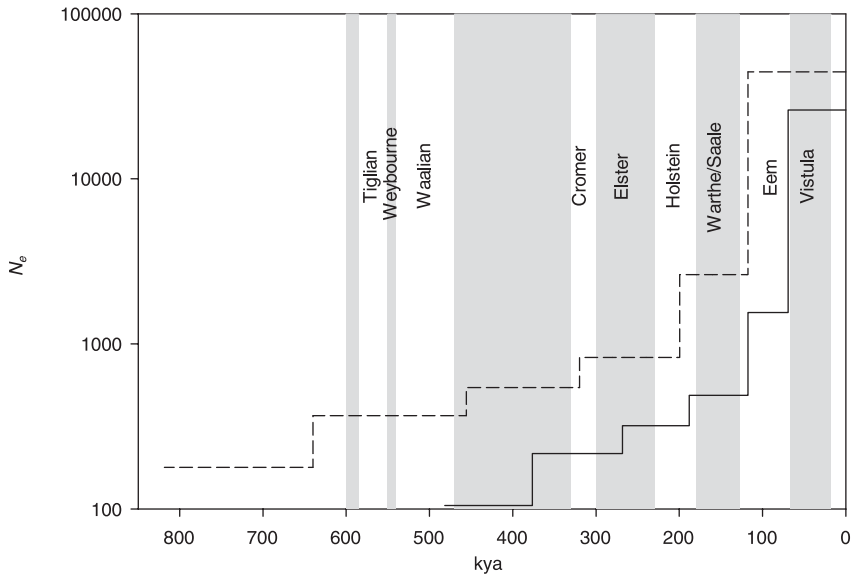

Fig. 4 Generalized skyline plot for *Fucus serratus* with the lower (dash, 2%/Myr) and upper (solid, 3.4%/Myr) boundaries of the molecular clock. The X axis represents the time before present (thousand years ago) and the Y axis (log) the estimated effective population size (N_e). Glacial (grey) and interglacial (white) periods are indicated.

from the Irish refugium via northern Scotland to Scandinavia (including the Skagerrak, Kattegat, and lower Baltic Seas) was dominated by one haplotype (h1) and the starlike topology of Cluster 1 indicates that the sweep was rapid (Figs 1 and 2). Based on an expansion rate of *c*. 0.5 km/year (Coyer *et al*. 2006b), recolonization of *F. serratus* from southwestern Ireland to the Orkney/Shetland Islands may have required as little as 2 000 years. We further hypothesize that *F. serratus* reached southern Norway between 15 000 years and 10 000 years ago, possibly in the area of Ålesund, which displayed the highest genetic diversity for Norway. From southern Norway, *F. serratus* required an additional *c*. 5000 years to reach the White Sea. Rapid expansion from a single recolonization wave into the Kattegat and the lower Baltic Seas is supported by finding mtDNA (*nad*11) heteroplasmy unique to the area (Coyer *et al*. 2004b), which was established as a marine environment *c*. 7500 years BP (Dawson 1992; Björck 1995).

Recolonization of *F. serratus* from the Brittany-Hurd Deep refugium could have commenced only after the Hurd Deep marine lake was reconnected to the sea *c*. 13 000 years ago (based on present bathymetry and predicted changes in sea level from Fleming *et al*. 1998). Lack of suitable rocky habitat northeastward along the southern coast of the North Sea and southward into the Bay of Biscay; along with lethally warm temperatures in the latter, undoubtedly explain the present distribution.

We further hypothesize that *F. serratus* expanded into the northern portion of the present Irish Sea, stopping at the northern coast of a land bridge that existed across the present Celtic Sea (between southern Ireland and Cornwall) (Lambeck 1996). It was not until the land bridge was breached that contact between the areas could occur with subsequent admixture between Cluster 1 and Cluster 2 in the central Irish Sea. As the English Channel opened and the North Sea filled from the south, the Brittany cluster (Cluster 2) moved eastward. The present shores of much of the North Sea consist primarily of soft substrates with only intermittent rocky substrates that are suitable for fucoids (e.g. Helgoland). Sampling from the eastern coast of the UK, which also consist of predominantly soft substrates is missing from our analysis, therefore the junction between the two haplotype clusters remains to be determined.

The distribution of haplotypes suggests that the Iberian refugium did not contribute to the recolonization of the rest of Northern Europe. Indeed, Iberian populations of *F. serratus* are now isolated and the shared haplotype between Spain and Ireland (Figs 1 and 2) is most likely a shared ancestral haplotype as the divergence between clusters predates the LGM.

Historical demography

Several glacial-interglacial periods have occurred since the mid-Pleistocene (Fig. 4), but the LGM undoubtedly has shaped the present day phylogeographical distribution of *F. serratus*. This does not imply, however, that *F. serratus* went through a strong bottleneck as a consequence of widespread extinction. The generalized skyline plot suggests that the largest expansion period for *F. serratus* occurred much earlier during the Eem Interglacial (128 000–67 000 years ago) (Fig. 4). Pre-LGM population expansion appears to be a common feature of coastal marine benthic species in the northeastern Atlantic (the red alga *P. palmata*, Provan *et al*. 2005; the soft bottom polychaetes *P. koreni* and *O. fusiformis*, Jolly *et al*. 2006). The Eem Interglacial followed the Warthe/Saale glaciation (128 000–180 000 years ago), which is thought to have had more widespread effects than

the LGM (Kellaway *et al*. 1975). Even earlier expansions, however, have been suggested for the common goby (536 000 years ago, Gysels *et al*. 2004) and the thornback ray *Raja clavata* (580 000–362 000 years ago, Chevolot *et al*. 2006).

The only previous attempt to derive a molecular-clock for marine heterokont macroalgae, was based on restriction fragment length polymorphism (RFLP) analysis of chloroplast DNA (cpDNA) and a biogeographical calibration of cpDNA divergence between northern and southern hemisphere populations of the giant kelp (Laminariales), *Macrocystis integrifolia* (see Druehl & Saunders 1992), closely related to *Fucus*. To our knowledge, our molecular clock for a multicellular heterokont is the first attempt based on direct DNA sequencing. We fully recognize that the clock's calibration, which is based on two prior estimates, is risky; yet the clock agrees well with proposed evolutionary scenarios. The 10–16 Myr divergence time between *A. nodosum* and *Fucus* is within the 7–38 Myr divergence time between the southern Hemisphere fucoid *Xiphophora* and all northern Hemisphere Fucaceae based on internal transcribed spacer (ITS) sequences (Serrão *et al*. 1999); and the 2.3–5.5 Myr divergence within *Fucus* is compatible with *Fucus* radiation in the North Atlantic after the opening of the Bering Strait, 4.1–7.4 Myr BP (Coyer *et al*. 2006a). Furthermore, the clock would have to be > 20%/Myr in order to reveal a post LGM expansion, a rate incompatible with *Fucus* evolutionary history. Although our clock must be interpreted with caution, it is possible that population expansion of *F. serratus* occurred before the LGM.

Conclusion

The greatest demographic expansion of *Fucus serratus* appears to have occurred during the Eem Interglacial period, 50 000–100 000 years before the LGM. At the time of the LGM, at least three glacial refugia existed and the legacy of each has differed considerably. The Iberian refugium was not important in postglacial expansion, whereas the southwestern Ireland refugium was as evidenced by its genetic signature being present in virtually all northern European populations. The Brittany (Hurd Deep) refugium was less important, presumably because of habitat constraints to expansion. The emerging picture of marine phylogeography in the European North Atlantic is complex, but remarkably consistent with respect to refugia among major benthic species (C. A. Maggs, unpublished). It is also clear that the LGM did not create a *tabulae rasa*, but was simply the latest event among many.

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