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Phylogeographic analysis of the red seaweed *Palmaria palmata* reveals a Pleistocene marine glacial refugium in the English Channel

JIM PROVAN*, REMI A. WATTIER† and CHRISTINE A. MAGGS*

*School of Biology and Biochemistry, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, †Equipe Ecologie-Evolution, UMR CNRS 5561 Biogéosciences, Université de Bourgogne, 6 Boulevard Gabriel, 21000 Dijon, France

Abstract

Phylogeography has provided a new approach to the analysis of the postglacial history of a wide range of taxa but, to date, little is known about the effect of glacial periods on the marine biota of Europe. We have utilized a combination of nuclear, plastid and mitochondrial genetic markers to study the biogeographic history of the red seaweed *Palmaria palmata* in the North Atlantic. Analysis of the nuclear rDNA operon (ITS1-5.8S-ITS2), the plastid 16S-*trnI-trnA*-23S-5S, *rbcL-rbcS* and *rpl12-rps31-rpl19* regions and the mitochondrial *cox2-3* spacer has revealed the existence of a previously unidentified marine refugium in the English Channel, along with possible secondary refugia off the southwest coast of Ireland and in northeast North America and/or Iceland. Coalescent and mismatch analyses date the expansion of European populations from approximately 128 000 BP and suggest a continued period of exponential growth since then. Consequently, we postulate that the penultimate (Saale) glacial maximum was the main event in shaping the biogeographic history of European *P. palmata* populations which persisted throughout the last (Weichselian) glacial maximum (c. 20 000 BP) in the Hurd Deep, an enigmatic trench in the English Channel.

Keywords: *cox2-3*, glacial refugia, ITS, *Palmaria palmata*, phylogeography, plastid

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Introduction

The present day distribution of the flora and fauna of Europe has been primarily shaped by climatic changes during the Quaternary period. Extended episodes of glaciation interspersed with shorter, warmer interglacial periods led to southward contractions in the ranges of the majority of species that subsequently persisted throughout the glacial maxima in one or more refugia (for reviews see Hewitt 1999, 2000). Fossil and palynological data have, to an extent, facilitated the analysis of potential refugia and recolonization routes in many plant and animal species but, until recently, it has generally not been possible to study the biogeographic history of organisms with a limited or nonexistent fossil record.

Phylogeography, the study of the geographic distribution of genetic lineages within species, has provided a new approach to the analysis of the postglacial history of a wide

range of taxa (for reviews see Taberlet 1998; Taberlet *et al.* 1998). Phylogeographic studies in European animals (e.g. Cooper *et al.* 1995; Seddon *et al.* 2001; Rokas *et al.* 2003) and plants (e.g. Dumolin-Lapègue *et al.* 1997; Grivet & Petit 2002; Lumaret *et al.* 2002) have confirmed the existence of three main terrestrial refugia during the last Pleistocene glaciation (115 000–10 000 BP) located in the Iberian, Italian and Balkan peninsulas. With a few exceptions (e.g. Wares & Cunningham 2001; Coyer *et al.* 2003; Luttikhuisen *et al.* 2003; Nikula & Vainola 2003; Olsen *et al.* 2004), however, comparatively little research has been carried out into the effect of the glaciation on the marine biota of Europe. We have used a phylogeographic approach to study the postglacial distribution of genetic lineages in the red seaweed, *Palmaria palmata*.

P. palmata was selected because of its wide distribution, as it is harvested commercially in part of its range and because the postglacial history of a related species, *Palmaria mollis*, has been studied in the northeast Pacific (Lindstrom *et al.* 1997). It is an arctic-cold temperate North Atlantic species, present on both European and North

Correspondence: Jim Provan, Fax: +44 28 9033 5877;

E-mail: J.Provan@qub.ac.uk

American coasts. In Europe, the southern and northern limits are Portugal (40° N latitude) and Spitzbergen (80° N), respectively (Irvine 1983; Lüning 1990). In North America, it ranges from Arctic Canada to Long Island, New York. Present day *P. palmata* is endemic to the North Atlantic and its basal position in previous phylogenetic analyses suggests that Palmariaceae probably originated in the Atlantic and that an ancestor of the other members of the family subsequently migrated to the Pacific, leaving *P. palmata* as the sole Atlantic representative (Lindstrom *et al.* 1996). In this study we have analysed samples of *P. palmata* from its entire range using markers specific to the nuclear, mitochondrial and plastid genomes. The aim of our study was to determine the phylogeographic structure of *P. palmata* in the North Atlantic in order to reconstruct its periglacial history, including identification of any potential glacial refugia.

Materials and methods

Sampling and DNA extraction

A total of 264 individuals were sampled from 42 locations along the European and the northeast North American coasts. For the analysis of geographic distribution of genetic variation, locations were grouped into 10 areas according to both geographic proximity and recognized hydrogeographic regimes (Table 1). DNA was isolated using either the method of Wattier *et al.* (2000) or the QIAGEN DNeasy Plant Mini Kit.

PCR-RFLP analysis

Five pairs of polymerase chain reaction (PCR) primers were used in total: two to amplify the nuclear rDNA operon (ITS1-5.8S-ITS2) and three to amplify two regions of the plastid genome (Table 2). The first pair of plastid-specific primers amplified a 2177-bp fragment including the 16S rDNA, *trnI*, *trnA* and approximately the first 450 bp of the 23S rDNA. The second pair amplified a 2519-bp product including the remainder of the 23S rDNA and approximately the first 100 bp of the 5S rDNA. The third pair amplified a 1984 bp fragment corresponding to the *rbcL* and *rbcS* genes and the 75 bp intergenic spacer. PCR was carried out in a final volume of 100 µL containing 200 ng DNA, 20 pmol of each primer, 1× PCR reaction buffer [20 mM Tris-HCl (pH 8.4), 50 mM KCl], 1.5 mM MgCl₂, 200 µM dNTPs and 1 U *Taq* polymerase (Gibco BRL). Reactions were carried out on a Perkin-Elmer 480 thermal cycler using the following parameters: initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 1 min, extension at 72 °C for 3 min (nuclear ITS and plastid rDNA) or 1 min (RuBisCO); final extension at 72 °C for 5 min. Eight microlitre aliquots of each PCR product

were digested with the following 11 enzymes according to manufacturer's recommendations: *AluI*, *AvaII*, *BsmAI*, *DdeI*, *HaeIII*, *HhaI*, *HinfI*, *HpaII*, *RsaI*, *Sau3AI* and *TaqI*. Digested PCR products were resolved on 2.5% agarose gels and visualised by ethidium bromide staining.

Plastid sequencing

A product, approximately 500 bp from the *rpl12-rps31-rpl9* region of the plastid genome, was amplified using the universal rhodophyte primer pair URP3F/R described in Provan *et al.* (2004). PCR was carried out on an MWG Primus thermal cycler using the following parameters: initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR was carried out in a total volume of 20 µL containing 100 ng genomic DNA, 10 pmol of forward primer, 10 pmol of reverse primer, 1× PCR buffer [5 mM Tris-HCl (pH 9.1), 1.6 mM (NH₄)₂SO₄, 1.5 µg/mL BSA], 2.5 mM MgCl₂, 200 µM dNTPs and 1.0 U *Taq* polymerase (Genetix). 10 µL each PCR product was resolved on a 2% agarose gel and visualized by ethidium bromide staining and the remaining 10 µL sequenced commercially (Macrogen).

Mitochondrial sequencing

The *cox2-3* spacer region of the mitochondrial genome was amplified using the primers described by Zuccarello *et al.* (1999). PCR, electrophoresis and sequencing were carried out as described previously.

Data analysis

For both the nuclear and the plastid PCR-RFLP data, polymorphisms were scored as unordered binary characters and combined to define haplotypes. Diversity values (*H*) based on haplotype frequencies were calculated using Nei's index (Nei 1987). Analysis was carried out using the ARLEQUIN software package (version 2.0; Schneider *et al.* 2000).

Sequences were aligned using the CLUSTALW program in the BIOEDIT software package and a minimum-spanning tree constructed manually. Diversity values based on haplotype frequencies were calculated as above. In addition, nucleotide diversity values (π ; Nei 1987) were calculated and a pairwise mismatch distribution analysis (Rogers & Harpending 1992) and Fu & Li's (1993) *D** test for departure from neutrality were carried out to test for population expansion. The last three analyses were carried out using the DNASP software package (version 3.99; Rozas & Rozas 1999).

To date the population expansion, the mutation rate used for the *cox2-3* region (0.55% / Myr) was the average of the values for red algae quoted by Zuccarello & West

Sampling location		N	Collector*
Boreal northeast Atlantic			
1 Keflavik, Iceland	KEF	1	CAM
2 Kaldbak, Faero Islands	KAL	6	RN
3 Trondheim, Norway	TRO	14	RH
4 Finnøy, Norway	FNY	1	JRu
North Sea			
5 Trønderøy, Norway	TRY	1	JRu
6 St Andrews, Scotland	STA	5	LP
7 Flamborough, England	FLA	5	FB
Temperate Northeast Atlantic			
8 Camas Malag, Scotland	CAM	5	SS
9 Lochcarron, Scotland	LOC	6	SS
10 Rattray Head, Scotland	RAT	5	SS
11 Fanad Head, Republic of Ireland	FAN	7	CAM
12 St John's Point, Co. Donegal, Republic of Ireland	SJP	10	CAM
Irish Sea			
13 Donaghadee, Northern Ireland	DON	18	RAW
14 Isle of Man, UK	IOM	5	UB
Galway Bay			
15 Mace Head, Republic of Ireland	MAC	2	SK
16 Spiddal, Republic of Ireland	SPI	16	SK
17 Salthill, Republic of Ireland	SAL	8	CAM
18 Finavarra, Republic of Ireland	FIN	22	SK
Celtic Sea			
19 Roscarberry Bay, Republic of Ireland	RCB	8	CAM
20 Rosslare, Republic of Ireland	ROS	5	CAM
21 Dale Point, Wales	DAL	5	CAM
English Channel			
22 Plymouth, England	PLY	5	FB
23 Kimmeridge, England	KIM	5	GB
24 Isle of Wight, England	IOW	7	FB
25 Cap Gris-Nez, France	CGN	4	FL
26 Luc sur Mer, France	LSM	4	A-MR
27 Cherbourg, France	CHE	5	CD
28 Cap de la Hague, France	CDH	2	OD
29 Deleg-St Anne du Portzic-Brest, France	DEL	5	EAG
Bay of Biscay			
30 Concarneau, France	CON	4	YLG
31 Belle Ile en Mer, France	BIM	1	RK
32 La Nora, Spain	LAN	5	JRi
33 Playa del Lagoa Lugo, Spain	PLL	3	JC
Portugal			
34 Vila Praia de Ancora, Portugal	VPA	2	RM
35 Melado, Portugal	MEL	2	RM
36 Praia de Montedor, Portugal	PRA	6	IPS
37 Castela do Neiva, Portugal	CDN	2	RM
Northeast North America			
38 Long Island, USA	LON	12	LL
39 Newfoundland, Canada	NEW	8	AW
40 Bay of Fundy, Canada	BOF	10	TC
41 Halifax, Canada	HAL	13	GS
42 Baffin Island, Canada	BAF	1	GS

Table 1 *Palmaria palmata* samples used in this study

*RN, Ruth Nielsen; JRu, Jan Rueness; LP, L. Poole; FB, Francis Bunker; SS, Sue Scott; CAM, Christine Maggs; RAW, Remi Wattier; UB, U. Bates; SK, Stefan Kraan; GB, G. Blunden; A-MR, A-M. Rusig; CD, C. Dupre; OD, O. Dargent; EAG, A. Ar Gall; YLG, Yves Le Gall; RK, R. Kaas; JRi, Jose Rico; JC, Juan Correa; RM, Ricardo Melo; LL, Larry Liddle; IPS, Isobel Pinto Sousa; AW, Alan Whittick; TC, Thierry Chopin; GS, Gary Saunders.

Genome	Name	Target	Sequence
Nuclear	ITSAF	ITS1-5.8S	TTTCCGTAGGTGAACCTGC
	ITSAR		ATATGCTTAAGTTCAGCGGGT
	ITSBF	5.8S-ITS2	AACTTAAAGGAATTGACGGAAG
	ITSBR		GGTCCGTGTTCAGACGGG
Plastid	PPrDNA1F	16S- <i>trnI-trnA</i> -23S	CATGGAGAGTTTGATCCTGGCT
	PPrDNA1R		CTGATTCACACGGGATTCACG
	PPrDNA2F	23S-5S	ACCGATAGTGAACAGTACCGC
	PPrDNA2R		CAGTATCATTGCCGCTGTAGTG
	PPrbcLSF	<i>rbcL-rbcS</i>	GAGGAATACATGTCTCAATCCG
	PPrbcLSR		CTAGTAACGAGAGCCTTCAGG

Table 2 PCR-RFLP primers used in this study

(2002). The time since the most recent expansion (t) was calculated from the mismatch distribution analysis parameter τ using the equation $\tau = 2ut$ (Rogers & Harpending 1992), where u is the per-nucleotide mutation rate (5.5×10^{-9}) multiplied by the sequence length (389 bp). Values of τ and 95% confidence limits were calculated using the ARLEQUIN software package (version 2.0; Schneider *et al.* 2000). In addition, population growth parameters were calculated using the Metropolis-Hastings Monte Carlo algorithm implemented in the FLUCTUATE software package (Kuhner *et al.* 1998). The analysis used 10 short chains of 4000 steps each and five long chains of 20 000 steps each with a sampling increment of 20 to jointly estimate the parameters Θ (equal to $2N\mu$, where N is the effective population size and μ is the mutation rate) and g (exponential growth parameter). A graph of change in relative effective population size (N_{rel}) over time was generated from the equation $t = \ln(N_{rel})/\mu g$ as described by Wares & Cunningham (2001), e.g. the time at which the population was 50% of its current size was obtained by calculating $t = \ln(0.5)/\mu g$. Neither of the above analyses (mismatch or Metropolis-Hastings Monte Carlo) was carried out for the plastid *rpl12-rps31-rpl9* region because of the comparative lack of phylogenetic structure.

Results

Levels and patterns of genetic diversity

Nuclear and plastid PCR-RFLP data were obtained for the full set of 264 individuals. Two mutations were detected in the nuclear ITS1-5.8S-ITS2 locus using the enzymes *Ava*II and *Bsm*AI, giving rise to three distinct haplotypes. One of these (N-3) was found in all individuals from the four northeast North American populations and in the Iceland population, whilst another (N-2) was fixed in the four Portuguese populations. The final haplotype (N-1) was fixed in the remaining European populations (Table 3).

A total of four mutations were detected in the two plastid regions surveyed: PPrDNA2F/R + *Hae*III ($\times 2$), PPrbcLSF/R + *Alu*I and PPrbcLSF/R + *Dde*I. Combining

Table 3 Nuclear PCR-RFLP haplotypes

Region	Haplotype			
	N-1	N-2	N-3	<i>N</i>
Boreal northeast Atlantic	21	—	1	22
North Sea	11	—	—	11
Temperate northeast Atlantic	36	—	—	36
Irish Sea	23	—	—	23
Galway Bay	48	—	—	48
Celtic Sea	18	—	—	18
English Channel	37	—	—	37
Bay of Biscay	13	—	—	13
Portugal	—	12	—	12
North America	—	—	44	44
Total	207	12	45	264

the four as unordered characters gave six haplotypes, designated P-1 to P-6 (Table 4 and Fig. 1). Haplotypes P-1 and P-2 were the most common, being found in six and seven of the 10 regions, respectively, and accounting for 113 and 97 out of 264 (80%) of the individuals studied. Haplotype P-3 was the next most common (43 individuals) but was only found in the four Galway Bay populations, two of which were fixed for that haplotype. The remaining three haplotypes were only found in the English Channel populations at relatively low frequency. In general, haplotype P-1 was found mostly in populations to the north and northeast of the English Channel, as well as being fixed in all five northeast North American populations, whilst haplotype P-2 was the dominant haplotype in populations to the northwest and southwest of the English Channel, with the exception of the Galway Bay populations. Only four of the 10 regions displayed any within-region variation (boreal northeast Atlantic, temperate northeast Atlantic, Galway Bay and English Channel). The boreal northeast Atlantic, temperate northeast Atlantic and Galway Bay regions each harboured two haplotypes whereas the English Channel was characterised by five haplotypes (P-1, P-2, P-4, P-5, P-6). Haplotype diversity values in variable regions ranged from 0.191 ± 0.070 (Galway Bay) to 0.695 ± 0.048 (English Channel).

Table 4 Plastid PCR-RFLP haplotypes and diversity values (standard deviation in parenthesis)

Region	Haplotype						N	H
	P-1	P-2	P-3	P-4	P-5	P-6		
Boreal northeast Atlantic	16	6	—	—	—	—	22	0.410 (0.072)
North Sea	11	—	—	—	—	—	11	—
Temperate northeast Atlantic	10	26	—	—	—	—	36	0.413 (0.068)
Irish Sea	23	—	—	—	—	—	23	—
Galway Bay	—	5	43	—	—	—	48	0.191 (0.070)
Celtic Sea	—	18	—	—	—	—	18	—
English Channel	10	17	—	1	7	2	37	0.695 (0.048)
Bay of Biscay	—	13	—	—	—	—	13	—
Portugal	—	12	—	—	—	—	12	—
North America	44	—	—	—	—	—	44	—
Total	113	97	43	1	7	2	264	0.489 (0.092)

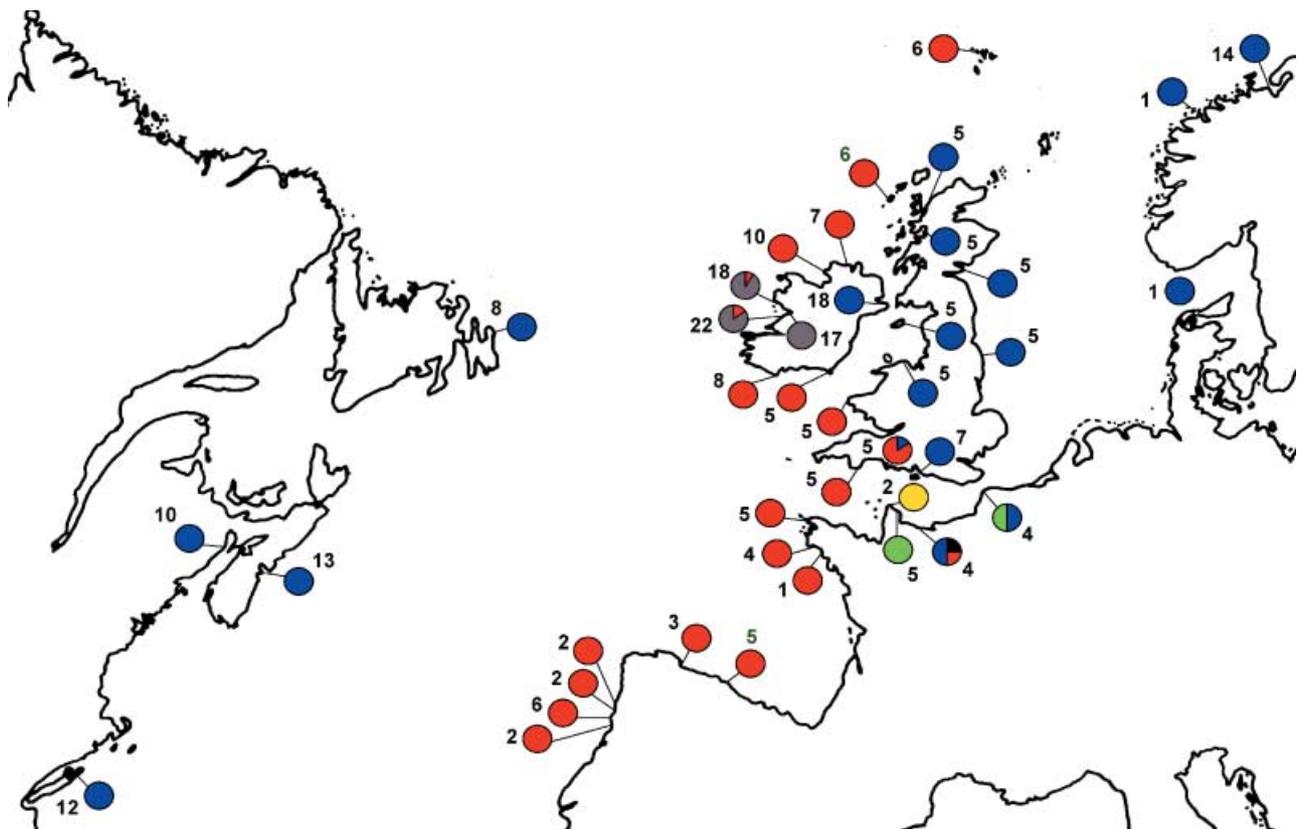


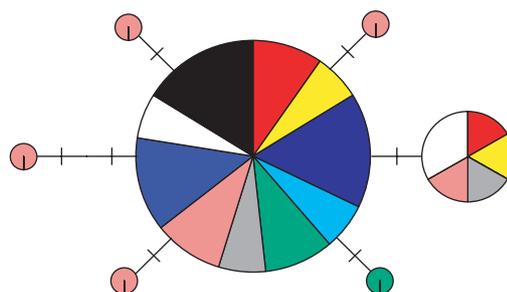
Fig. 1 Distribution of plastid PCR-RFLP haplotypes. Numbers represent sample sizes for each population (see Table 1). Not shown: Iceland and Baffin, both blue (haplotype P-1).

The sequences of the *rpl12-rps31-rpl9* region of the plastid genome and the mitochondrial *cox2-3* spacer were elucidated for one individual from each of the 42 locations sampled. The plastid sequences yielded an alignment of 497 bp of which seven sites were polymorphic. No insertion-deletion mutations were observed. Seven haplotypes (designated P-I to P-VII; Table 5 and Fig. 2; GenBank

Accession nos AY826505–AY826514) were observed and the distribution and frequency of haplotypes were largely congruent with the results of the plastid PCR-RFLP analysis. Haplotype P-I was found in all regions and in 31 of the 42 individuals sequenced, with haplotype P-II being the only other haplotype represented more than once (six times). As in the PCR-RFLP analysis, the English Channel

Table 5 Plastid sequencing haplotypes and diversity values (standard deviation in parentheses)

Region	Haplotype							N	π
	P-I	P-II	P-III	P-IV	P-V	P-VI	P-VII		
Boreal northeast Atlantic	3	1	—	—	—	—	—	4	0.0010 (0.0005)
North Sea	2	1	—	—	—	—	—	3	0.0013 (0.0006)
Temperate northeast Atlantic	5	—	—	—	—	—	—	5	—
Irish Sea	2	—	—	—	—	—	—	2	—
Galway Bay	3	—	1	—	—	—	—	4	0.0010 (0.0005)
Celtic Sea	2	1	—	—	—	—	—	3	0.0013 (0.0006)
English Channel	3	1	—	1	1	1	1	8	0.0030 (0.0008)
Bay of Biscay	4	—	—	—	—	—	—	4	—
Portugal	2	2	—	—	—	—	—	4	0.0013 (0.0004)
North America	5	—	—	—	—	—	—	5	—
Total	31	6	1	1	1	1	1	42	0.0011 (0.0003)

**Fig. 2** Minimum spanning tree showing relationships between plastid *rpl12-rps31-rpl9* haplotypes. Circle sizes are approximately proportional to haplotype frequency.

was the most haplotype-rich region, with six of the seven haplotypes being present. Again, as for PCR-RFLP, a third haplotype (P-III) was found localised in the Galway Bay region. Because of small sample sizes, haplotype diversity values were not significantly different from each other (data not shown) but the nucleotide diversity (π) for the English Channel (0.0030 ± 0.0008) was significantly higher than the next highest regional values (North Sea, Celtic Sea and Portugal: 0.0013 ± 0.0006 , 0.0013 ± 0.0006 and 0.0013 ± 0.0004 , respectively).

Alignment of the mitochondrial *cox2-3* region revealed 13 polymorphic sites over a total length of 389 bp and gave rise to 13 distinct haplotypes (M-1 to M-13; Table 6 and Fig. 3). As in the previous organellar analyses, one dominant haplotype (M-1; GenBank Accession nos AY828190–AY828202) was found in majority of the populations studied and accounted for 26 of the 42 individuals analysed. One major difference revealed by the mitochondrial data, however, was the presence in the northeast North American samples of three endemic haplotypes (M-11 to M-13) which differed from all European haplotypes by at least two mutations (Fig. 3). Haplotype M-11 was also

found in the Iceland sample studied. Diversity values in populations that displayed variation ranged from 0.0013 ± 0.0007 (Galway Bay and Bay of Biscay) to 0.0051 ± 0.0017 (boreal Northeast Atlantic) but, unlike in the two previous analyses, no one population displayed a significantly higher diversity value than the others.

Patterns of historical demography

The minimum spanning trees for both the plastid *rpl12-rps31-rpl9* region (Fig. 2) and the mitochondrial *cox2-3* spacer (Fig. 3) largely exhibited star-like topologies, with the majority of the individuals studied possessing the dominant central haplotype in both cases. Such a topology is usually indicative of a recent population expansion and although the graphs of pairwise nucleotide differences did not show a clear unimodal distribution of mismatches in either case (data not shown), the observed raggedness values were not significantly different from the mismatch distribution expected from a sudden expansion (data not shown). Population demography statistics based on data from the mitochondrial *cox2-3* region are given in Tables 7 and 8.

Table 6 Mitochondrial sequencing haplotypes and diversity values (standard deviation in parentheses)

Region	Haplotype													N	π
	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8	M-9	M-10	M-11	M-12	M-13		
Boreal northeast Atlantic	2	1	—	—	—	—	—	—	—	—	1	—	—	4	0.0051 (0.0017)
North Sea	1	—	1	1	—	—	—	—	—	—	—	—	—	3	0.0034 (0.0011)
Temperate northeast Atlantic	3	1	—	—	—	1	—	—	—	—	—	—	—	5	0.0021 (0.0008)
Irish Sea	1	—	—	—	—	1	—	—	—	—	—	—	—	2	0.0026 (0.0013)
Galway Bay	3	—	—	—	—	—	1	—	—	—	—	—	—	4	0.0013 (0.0007)
Celtic Sea	3	—	—	—	—	—	—	—	—	—	—	—	—	3	—
English Channel	6	—	—	—	—	—	—	1	1	—	—	—	—	8	0.0017 (0.0008)
Bay of Biscay	3	—	—	—	—	—	—	—	—	1	—	—	—	4	0.0013 (0.0007)
Portugal	4	—	—	—	—	—	—	—	—	—	—	—	—	4	—
Northeast North America	—	—	—	—	—	—	—	—	—	—	2	2	1	5	0.0026 (0.0009)
Total	26	2	1	1	1	1	1	1	1	1	3	2	1	42	0.0029 (0.0006)

Table 7 Population demographic parameters based on mismatch analysis of *cox2-3* sequences

Sample	τ		Generations since expansion			u	D*	
	Value	95% confidence		Value	95% confidence			
		Lower	Upper		Lower			Upper
All	1.949	0.217	5.714	455 373	50 701	1 335 046	2.140 × 10 ⁻⁶	-2.818, P < 0.05
Europe (excl. Iceland)	0.732	0.002	1.963	171 028	467	458 645	2.140 × 10 ⁻⁶	-2.910, P < 0.05
North America and Iceland	1.280	0.000	2.911	299 065	0	680 140	2.140 × 10 ⁻⁶	-0.710, NS

Table 8 Population demographic parameters based on Metropolis-Hastings Monte Carlo coalescent analysis of *cox2-3* sequences

Sample	Θ (no growth)	Θ (expansion)	g
All	0.0138	0.1165 ± 0.0133	2311 ± 114
Europe (excl. Iceland)	0.0086	0.1478 ± 0.0155	5251 ± 156
North America and Iceland	0.0029	0.0378 ± 0.0260	10 000 (± 1918)

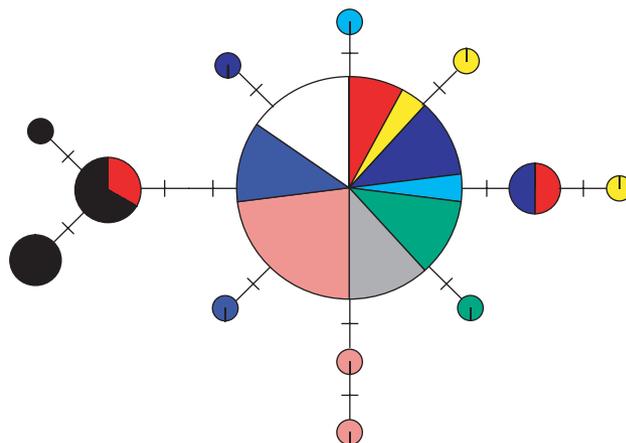


Fig. 3 Minimum spanning tree showing relationships between mitochondrial *cox2-3* haplotypes. Circle sizes are approximately proportional to haplotype frequency.

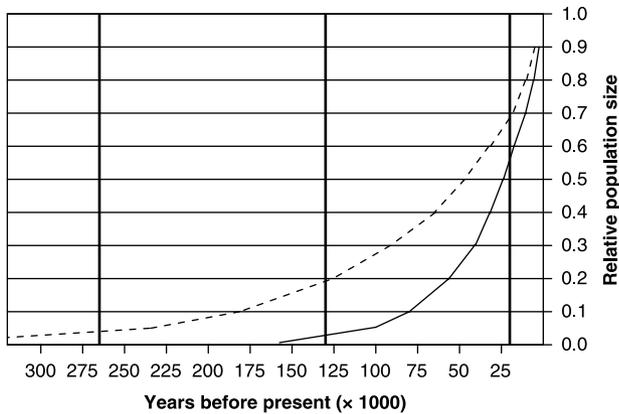


Fig. 4 Graph of change in effective population size over time based on Metropolis-Hastings Monte Carlo coalescent analysis. The solid line represents the European samples only whilst the dotted line represents the complete sample. Heavy vertical bars correspond to glacial maxima.

The values of F_u and Li's D^* were all negative (range: -0.710 to -3.128) and generally highly significant ($P < 0.05$ in all cases except the North American sample, probably because of small sample size), suggesting an excess of rare haplotypes relative to that expected in a population at equilibrium and also indicating rapid population expansions.

Because the variation observed in the *cox2-3* region suggested separate recolonization events in Europe and northeast North America, the value of t was calculated for the European and northeast North American/Icelandic populations separately using the equation $\tau = 2ut$ (Rogers & Harpending 1992) for t , with values of τ and u as shown in Table 7. This suggested that the expansion of the complete sample occurred 455 373 generations ago and separate values calculated for the European and northeast North American/Icelandic populations suggested that the expansion of the European populations occurred 171 028 generations ago whilst the northeast North American/Icelandic populations expanded 299 065 generations ago. The coalescent analysis of change in effective population sizes (Table 8 and Fig. 4) suggests that the European populations were *c.* 1% of their present effective population size 159 456 BP (SD 154 855–164 338 years BP) and that the whole sample reached similar levels 362 213 BP (SD 345 280–381 112).

Discussion

For organisms with no fossil record, the analysis of genealogical lineages of extant taxa provides the only method for the reconstruction of biogeographic history. We have used such an approach to study the biogeography of *Palmaria palmata* in the North Atlantic and North Sea during and after the last Pleistocene glaciation. Our

findings suggest the existence of a major glacial refugium in the English Channel region, with possible secondary refugia off the western coast of Ireland and the eastern coast of North America and in Iceland.

Both the PCR-RFLP and plastid sequencing analyses revealed the highest levels of haplotype and nucleotide diversity in the English Channel. Furthermore, there were several haplotypes found in this region that were not detected elsewhere in Northern Europe or northeast North America. Such patterns are characteristic of glacial refugia and resemble those detected in previous plant (Konnert & Bergman 1995; Demesure *et al.* 1996; Dumolin-Lapègue *et al.* 1997; Ferris *et al.* 1998; King & Ferris 1998) and animal (Wallis & Arntzen 1989; Cooper *et al.* 1995; Santucci *et al.* 1998) studies that highlighted the existence of the Iberian, Italian and Balkan refugia. A previous study on the large brown seaweed *Fucus serratus* using microsatellites reported higher levels of genetic diversity in populations from Brittany than in other European populations (Coyer *et al.* 2003) and a similar result was also found in a parallel microsatellite study on another furoid, *Ascophyllum nodosum* (Stam *et al.* 2001). These findings, like the results of the present study, are consistent with the existence of a refugium in the English Channel. In addition, a study on the common goby (*Pomatoschistus microps*) by Gysels *et al.* (2004) suggested a refugium in southern North Sea but their data could not rule out completely the possibility of this refugium being located in the English Channel.

Although the English Channel was south of the maximum extent of the late Weichselian (25 000–10 000 BP) ice sheets, at the last glacial maximum lowstand when the sea level was 135 m below present, the English Channel was dry land (Frenzel *et al.* 1992; Lambeck *et al.* 2002). Geophysical (seismic and bathymetric) analyses, however, have highlighted the existence of an enigmatic trench known as the Hurd Deep, a 150-km long depression about 100 m deeper than the adjoining sea floor (Smith 1985; Lericolais *et al.* 1995, 2003). Although the Hurd Deep was formed as part of the Channel palaeoriver system, it is most likely that it was formed during the incision phase associated with an earlier regression and that during the last glacial maximum (and possibly during previous glacial maxima) it persisted as a marine lake until the sea levels rose again around 10 000 BP. Recent modelling reconstructions (Sarnthein 2001) suggest that sea temperatures near the shelf in the region of the English Channel were 1–2 °C in winter, rising to 5–6 °C in summer. These conditions are almost identical to those existing today in Svalbard (Luning 1990), where *P. palmata* is extremely common. Although there may have been some freshwater input into this lake, it is believed that the major rivers of northern France did not join the Hurd Deep but instead turned westwards (Antoine *et al.* 2003). Even if there had been substantial levels of freshwater input, it is likely that the lake could have remained saline

below a certain depth and it has been shown that *P. palmata* can grow at low temperatures and irradiance (Sagert & Schubert 2000). Likewise, *Fucus* spp. can become subtidal under challenging conditions such as those found in the Baltic today (Makarov *et al.* 1999). The Hurd Deep has bedrock walls, in contrast to the sedimentary bottom of much of the English Channel. During the lowstand, this substratum would have been suitable for *P. palmata*, which is confined to rocky coasts and absent from sedimentary coastlines such as eastern England. Consequently, we suggest that Channel palaeoriver deeps formed a refugium for several species of benthic macroalgae during the last glacial maximum.

The distribution of the PCR-RFLP haplotypes also suggests two main routes of postglacial recolonization of Europe and the North Atlantic by *P. palmata*. The dominance of haplotype P-1 in the northern/eastern populations and the dominance of haplotype P-2 in the western populations indicate separate northwards and westward recolonization routes from the English Channel. This geographically structured pattern of recolonization may be a result largely of the poor dispersal ability of *P. palmata*, as suggested for *Palmaria mollis* in the northeast Pacific (Lindstrom *et al.* 1997). Members of this genus have only a single spore-releasing phase in the life history (the diploid sporophyte) and the haploid spores released after meiosis are nonmotile and settle quickly, usually within 24 h (Van der Meer & Bird 1985). The minute females are fertilized by sperm, which lacks plastids, and are overgrown by the diploid sporophyte shortly afterwards. Drifting thalli would therefore represent the only possible means of long distance dispersal but this seems to occur rarely based on empirical evidence from the colonization of the volcanic island of Surtsey after its eruption in 1963 off the southwest coast of Iceland. The nearest source of algae is the Vestmannaeyjar archipelago, 2.7 nautical miles from Surtsey at its closest point. The kelp *Laminaria hyperborea* had become established by 1968 and in 1997, 34 years after the eruption, 76 species had been recorded. *P. palmata*, however, which is common in southern Iceland both intertidally and on subtidal *L. hyperborea* stipes, had not been observed despite the presence of its favoured substratum (Irvine 1983; Jónsson & Gunnarsson 2000).

A second potential refugium may have existed off the west coast of Ireland around the Galway Bay area. This is highlighted by the occurrence of an endemic haplotype in both the plastid PCR-RFLP and sequencing analyses. Whilst it is not obvious why this region should harbour a refugium, the theory has been previously proposed for several terrestrial species, including Scots pine (*Pinus sylvestris*: Sinclair *et al.* 1998) and moss mites (Oribatida; Seyd 1992). It is possible that the southwest of Ireland lay beyond the limits of both the ice sheets and permafrost during the last glacial maximum (Frenzel *et al.* 1992).

Similarly, the presence of an endemic, fixed nuclear PCR-RFLP haplotype in the North American and Icelandic populations along with the existence of a group of phylogenetically discordant mitochondrial lineages suggest that both Iceland and the Atlantic coast of North America were recolonized from a separate refugium from that responsible for the recolonization of the rest of Europe. Wares & Cunningham (2001) also found a close association between Icelandic and North American haplotypes in the barnacle *Semibalanus balanoides*, as did Dahlgren *et al.* (2000) in a study on the ocean quahog (*Arctica islandica*). Although a unique nuclear PCR-RFLP haplotype was also found in all the Portuguese individuals studied, the lack of one or more corroborating haplotypes in the other analyses (that are found in the Galway Bay and northeast North American/Icelandic samples) suggests that this may be more likely to represent a recent, geographically localised mutation rather than a signature of a further refugium.

The significantly negative values of the Fu and Li's D^* test (with the exception of the northeast North American/Icelandic samples), the star-like genealogy of the minimum-spanning trees and the mismatch analysis are all consistent with recent population expansions. Although the 95% confidence intervals for the times since population expansion based on the mismatch analysis are large, the calculated values do correspond in general to significant glacial events. The value of 171 028 generations calculated from the mitochondrial sequence data for the European samples indicates an expansion of European *P. palmata* populations around 128 271 BP, based on an average generation time of 0.75 y (1–2 generations per year, M. Edwards, personal communication). This figure is broadly in agreement with those from the coalescent analysis (Fig. 4), which suggests that at the time of the Saale glacial maximum around 128 000 BP, the effective population size of European *P. palmata* was around 2% to 3% of current effective population size after which the populations experienced a period of continuous exponential growth until present day levels were reached. These findings therefore suggest that the penultimate, rather than the most recent, glacial maximum (c. 20 000 BP) was the major event shaping the present-day population demography of European populations. Taken together, our results would appear to confirm that European *P. palmata* populations underwent a severe bottleneck during the Saale glacial maximum but survived the Weichselian glaciation in a series of refugia, most notably in the Hurd Deep in the English Channel.

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Jim Provan and Christine Maggs are lecturers in the School of Biology and Biochemistry at the Queen's University of Belfast. Their research is mostly focused on the population and evolutionary genetics of various plant and algal species (for more details, go to <http://www.qub.ac.uk/bb/provan/>). Following postdoctoral positions at QUB and Bangor (Wales), Remi Wattier is now lecturing at the University of Bourgogne.
