

Surfing the wave on a borrowed board: range expansion and spread of introgressed organellar genomes in the seaweed *Fucus ceranoides* L

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Abstract

For many taxa, introgression represents an important source of genetic variation, but the specific contexts allowing locally introgressed material to spread and largely replace native allelic lineages throughout a species range remain poorly understood. Recent demographic-genetic simulations of spatial expansions show that the stochastic surfing of alien alleles during range expansions may constitute a general mechanism leading to extensive introgression, but empirical evidence remain scarce and difficult to distinguish from selection. In this study, we report a compelling case of such a phenomenon in the estuarine alga *Fucus ceranoides*. We re-assessed the phylogenetic relationships among *F. ceranoides* and its marine congeners *F. vesiculosus* and *F. spiralis* using nuclear, mitochondrial and chloroplast sequence data, and conducted a mtDNA phylogeographic survey in *F. ceranoides*. Our phylogenetic analyses revealed a recent and asymmetric introgression of a single *F. vesiculosus* cytoplasm into *F. ceranoides*. The phylogeographic scope of introgression was striking, with native and introgressed mtDNA displaying disjunct distributions south and north of the English Channel. A putative Pleistocene climatic refugium was detected in NW Iberia, and the extensive and exclusive spread of the alien cytoplasm throughout Northern Europe was inferred to have occurred concurrently with the species post-glacial, northwards range expansion. This massive spread of a foreign organelle throughout the entire post-glacial recolonization range represents good empirical evidence of an alien cytoplasm surfing the wave of a range expansion and the first description of such a phenomenon in the marine realm.

Keywords: *Fucus ceranoides*, genetic surfing, organellar introgression, phylogeography, polyphyly, range expansion

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Introduction

The transfer of genetic material across species boundaries by hybridization and backcrossing, i.e. introgression, is both taxonomically widespread and consequential for the genetic make-up and evolution of species (Mallet 2005; Arnold *et al.* 2008). Reproductive barriers tend to become increasingly effective with time since divergence, but there is typically an extended period during and after

speciation when hybridization can still bridge genetic exchange between species (Price & Bouvier 2002; Bull *et al.* 2006). Introgression can be highly selective, however; genomic regions strongly affected by divergent selection can remain virtually isolated, whereas cross-adaptive and neutral alleles may flow more freely between species and eventually penetrate further into a foreign gene pool (Martinsen *et al.* 2001; Scotti-Saintagne *et al.* 2004). In particular, organellar genomes (mitochondrial and chloroplast) appear especially prone to introgression, and ambiguities in phylogenetic inference (e.g. polyphyly, cyto/nuclear conflict) based on this class of

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markers have been widely documented in molecular systematics (Rieseberg & Soltis 1991; Funk & Omland 2003; Chan & Levin 2005).

Although for many taxa organelle capture clearly represents an important source of intra-specific genetic variation (Dumolin-Lapegue *et al.* 1999; Jackson *et al.* 1999; Weisrock *et al.* 2005), the dynamic spatio-temporal contexts allowing locally introgressed haplotypes to spread and largely replace native allelic lineages throughout a species range remain poorly understood. Phylogeographic evidence has increasingly supported the view that the geographic scope of introgressed material often reflects particular aspects of the demographic history of the recipient taxa (Dorado *et al.* 1992; Berthier *et al.* 2006; Liston *et al.* 2007). Recent demographic-genetic simulations of spatial expansions (Currat *et al.* 2008) also suggest that during range expansions, driven for example by climatic changes or species introductions, expanding taxa are particularly amenable to be massively introgressed by alleles of locally established species, provided that interbreeding, even if relatively infrequent, occurs close to the leading edge of the expansion. Strong genetic drift at expanding margins caused by the random sampling of alleles propagated through successive founder events can result in drastic allele frequencies changes at the frontline of the expansion (Hallatschek *et al.* 2007; Excoffier & Ray 2008). Even if initially rare, particular alleles may happen to reach high frequencies by chance at the leading edge and eventually spread over vast geographic areas surfing the wave of the expansion (Edmonds *et al.* 2004; Klopstein *et al.* 2006; Excoffier & Ray 2008). In theory, allelic variants introgressing at expanding frontiers can surf as well, but empirical evidence for a role of such surfing phenomena in producing extensive introgression remains scarce. Here, we report a compelling case of extensive dissemination of an alien cytoplasm surfing the wave of a range expansion in the seaweed *Fucus ceranoides*.

Fucus ceranoides L. (horned wrack) is a cold-temperate, European endemic furoid alga that occurs in the upper parts of estuaries and similar habitats (e.g. coastal outflows) subjected to the influence of freshwater for part of each tidal cycle. This perennial, dioecious alga is distributed from the Mondego River in Portugal (40°N Lat., personal observation) to Northern Norway (70°N Lat.) and Iceland, being absent from the Baltic and most of the North Sea (Luning 1990). *F. ceranoides* belongs to a monophyletic *Fucus* clade that includes two additional NE Atlantic marine species, *F. vesiculosus* L. and *F. spiralis* L. (Serrao *et al.* 1999), which range in latitude from NW Africa to northern Norway and are also present along NW Atlantic coasts (Luning 1990). Despite the morphological, ecological and genetic distinctiveness of the three species when using nuclear mi-

cro-satellites (Billard *et al.* 2005), a detailed molecular phylogeny of the genus based on mtDNA (Coyer *et al.* 2006) could not resolve them. This marked incongruence among markers suggested a reticulate history of organelle exchange across species boundaries. Incomplete reproductive isolation and hybridization has been recently verified with nuclear markers for most *Fucus* species (Coyer *et al.* 2002a; Engel *et al.* 2005), but the phylogenetic relationships among *F. ceranoides* and its close relatives, and the extent and direction of genetic exchange still remain unclear.

We used a combination of molecular markers specific to the nuclear, mitochondrial and chloroplast genomes to infer the patterns of genetic exchange between *F. ceranoides* and its parapatric congeners, and to assess its consequences for the species intraspecific phylogeography. Our results revealed the historical introgression of *F. vesiculosus* organellar genomes into the *F. ceranoides* gene pool, and their extensive spread concurrently with the species' northwards, post-glacial range expansion. The pattern of introgression observed matches remarkably well the predictions of simulation models and provides clear empirical support for an alien cytoplasm surfing the wave of a range expansion.

Material and methods

Sampling

For the phylogenetic analysis, we used a small panel of individuals spanning the NE Atlantic distribution of *Fucus ceranoides* ($n = 10$), *F. spiralis* ($n = 12$) and *F. vesiculosus* ($n = 12$), with *F. serratus* as the outgroup (see Table S1, supporting information for sampling locations). Taxon discrimination was made by the authors or by the phycological experts that collected the specimens, based on the following morphological/habitat criteria: presence of vesicles for *F. vesiculosus* (mid shore); round hermaphroditic receptacles and absence of vesicles for *F. spiralis* (upper shore); and thalli thinning towards the apices, and with flattened crown-shaped receptacles for *F. ceranoides* (river margins inside estuaries). For the *F. ceranoides* phylogeographic survey, 21 locations ($n = 497$) were sampled from Portugal to Norway, covering most of the species distribution in Northeast Atlantic (Table 1). Populations were collected by the authors or kindly provided by local experts following a common sampling scheme. All collection sites were subject to tidal regimes and salinity fluctuations. At each site, 5–10 cm tips of apical vegetative tissue was excised from 24 individuals sampled along a 50–200-m linear transect or random walk; tissue samples were individually stored dehydrated in silica-gel crystals until DNA extraction.

Table 1 *Fucus ceranoides* mtIGS haplotype frequencies and nucleotide diversity (π) for each geographic region and sampling site

Region Population	ID	N	Clade	Shared haplotypes				Private haplotypes	π (%)
				A1	C1	I1	I9		
<i>NW Iberia</i>		96	<i>N</i>					0,891	
Viana do Castelo, Norte, PT	1	24	<i>N_A</i>	21	—	—	—	A2(2), A3	0,049
Ria de Noia y Muros, W Galicia, ES	2	24	<i>N_A</i>	17	—	—	—	A4(7)	0,087
Ria de A Coruna, N Galicia ES	3	24	<i>N_B</i>	—	—	—	—	B1(15), B2(4), B3(3), B4(2)	0,183
River Porcia, W Asturias, ES	4	24	<i>N_C</i>	—	20	—	—	C2(2), C3, C4	0,083
<i>Cantabrian Sea</i>		72	<i>N</i>					0,212	
Ria de Villaviciosa, E Asturias ES	5	24	<i>N_C</i>	—	17	—	—	C5(6), C6	0,096
Marismas de Santona, Cantabria, ES	6	24	<i>N_C</i>	—	—	—	—	C7(15), C8(9)	0,099
Bayonne, S Aquitaine, FR	7	24	<i>N_C</i>	—	21	—	—	C9(2), C10	0,049
<i>Brittany & English Channel</i>		94	<i>N,I</i>					2,168	
Anse de Saint Laurent, S Brittany, FR	8	24	<i>N_C</i>	—	22	—	—	C11, C12	0,067
Penze, N Brittany, FR	9	24	<i>I</i>	—	—	21	—	I2(3)	0,046
Southampton, S England, UK	10	22	<i>N_C</i>	—	22	—	—	—	
Gweek, SW England, UK	11	24	<i>I</i>	—	—	24	—	—	
<i>Ireland & Wales</i>		95	<i>I</i>					0,158	
Milford Haven, S Wales, UK	12	24	<i>I</i>	—	—	6	—	I3(18)	0,079
Caernarfon, N Wales, UK	13	24	<i>I</i>	—	—	1	22	I10	0,034
Cork, Cork, IE	14	23	<i>I</i>	—	—	21	—	I4, I5	0,035
Ramelton, Donegal, IE	15	24	<i>I</i>	—	—	22	—	I6, I7	0,034
<i>Northern UK & Norway</i>		140	<i>I</i>					0,125	
Oban, W Scotland, UK	16	22	<i>I</i>	—	—	1	15	I11(6)	0,102
Orkneys, N Scotland, UK	17	22	<i>I</i>	—	—	—	22	—	—
Seaton Sluice, NE England, UK	18	24	<i>I</i>	—	—	23	—	I8	0,017
Hardangerfjord, Hordaland, NO	19	24	<i>I</i>	—	—	24	—	—	—
Tromdheimsfjord, Nord-Trondelag, NO	20	24	<i>I</i>	—	—	24	—	—	—
Folda, Nordland, NO	21	24	<i>I</i>	—	—	12	9	I12(2), I13	0,154

PT, Portugal; ES, Spain; FR, France; IE, Ireland; UK, United Kingdom; NO, Norway.

DNA isolation, amplification and sequencing

Genomic DNA was extracted from approximately 6–10 mg dried tissue using the Nucleospin® Multi-96 plant kit (Macherey-Nagel Duren, Germany), according to the manufacturer's protocol. Forward and reverse primers for the *c.* 440–480-bp mitochondrial 23S/trnK intergenic spacer (mtIGS, F 5'-GTGCAAGAGCTGG-GAAGTTT-3'; R 5'-CCCAAATGTAGGCGTATTGG-3') were designed from the complete mitochondrial genome of *F. vesiculosus* (Oudot-Le Secq *et al.* 2006). To test for genealogical concordance, the mtIGS tree was contrasted against a chloroplast and nuclear phylogeny for the same panel of individuals. A fragment of the chloroplast *open reading frame* 501 (cpORF501, ~780 bp long), located between the genes *petA* and *psaJ*, was selected, and primers (F 5'-CCAAGTTTTGAAAA-GAAGCAA-3'; R 5'-TTGATAATGTTGTTGCGATTCA-3') were designed from the complete chloroplast genome of *F. vesiculosus* (Le Corquille *et al.* 2009). For the development of a suitable nuclear marker, several primer pairs were designed for cDNA contigs derived from

F. serratus/*F. vesiculosus* EST libraries (Pearson *et al.* 2010) and tested for amplification in genomic DNA. The primer set designed for a ~700-bp cDNA encoding a putative protein disulfide isomerase successfully amplified a single ~1450-bp intron-rich genomic DNA fragment in all species; new primers were designed to target a ~880-bp exon-primed, intron-crossing polymorphic region (nPDI, F 5'-CGCGGGTCGATTCTTCAC-3'; R 5'-GAACTCCACCATCACGTCCT-3'). Polymerase chain reactions were performed in 20 μ L total volume containing 1 \times GoTaq Flexi buffer (Promega), 2.0 mM (mtIGS and cpORF501) or 1.0 mM (nPDI) MgCl₂, 125 μ M each dNTP, 0.5 μ M each primer, 1 U GoTaq® Flexi DNA Polymerase (Promega), and 2 μ L of 1:100 diluted DNA template. An initial denaturation step (94 °C, 5 min) was followed by 35 cycles of 94 °C for 30 s, 58 °C (mtIGS and cpORF501) or 61 °C (nPDI) for 30 s and 72 °C for 1 min and a final extension step (72 °C, 10 min).

Laboratory procedures used in *F. ceranoides* mtIGS phylogeography were the same as described earlier. MtIGS, CpORF501 and nPDI amplicons were cleaned with ExoSap (Fermentas) and sequenced in an automated

capillary sequencer (Applied Biosystems, CCMAR Portugal). Sequences have been deposited in GenBank database under accessions nos. GQ385112-GQ385190.

Phylogenetic and phylogeographic analyses

Phylogenetic relationships among *Fucus spp.* were reconstructed using maximum likelihood and Bayesian inference. For each data set, Mr. ModelTest 1.1 (Posada & Crandall 1998; Nylander 2004) was run in PAUP* (Swofford 2000) and best-fit nucleotide substitution models were selected based on Akaike information criterion scores. Selected models were K80 (K2P), HKY85 + I and GTR for the nPDI, mtIGS and cpORF501 data sets, respectively. Bayesian analyses were performed using MrBayes (Ronquist & Huelsenbeck 2003). Two parallel Metropolis-coupled Markov chain Monte Carlo searches, each with four chains (3 'heated'), were run for 2×10^6 generations, sampling trees and parameters every 100 generations. For each data set, the number of substitution rates (Nst = 2/6), among-site rate variation (Rates = equal/propinv) and base frequency priors [Statefreqpr = Dirichlet (1,1,1,1)/Fixed(equal)] were set according to the substitution model selected, leaving the remaining options as default. Run length sufficiency was confirmed by inspecting the average standard deviation of split frequencies between runs and cold chains Log-likelihood stationarity. Based on the latter, 10^5 generations (1000 trees) were discarded as burn-in. The remaining 38 000 trees sampled were used to produce 50% majority-rule consensus trees and to calculate branch posterior probabilities. Maximum likelihood analyses were performed with Garli (Zwickl 2006). Ten independent searches were performed for each data set, and the similarity of the independent trees and lnL scores was confirmed. Nodal support was calculated using 1000 bootstraps. Trees were rooted with *F. serratus*.

The geographic distribution of native and introgressed mtIGS lineages (as a proxy for the entire cytoplasm) throughout *F. ceranoides* range was assessed. The genealogic relationships of mtIGS haplotypes were inferred using a statistical parsimony algorithm implemented in TCS 1.21 (Clement *et al.* 2000), and nucleotide diversity (π) within populations and geographic regions was calculated with DNASP (Rozas & Rozas 1999). The partitioning of genetic variation at different hierarchical levels was examined with molecular analyses of variance (AMOVA) in ARLEQUIN 3.1 (Schneider *et al.* 2000). Components of genetic variance were computed by grouping populations according to their mtIGS clade (native or introgressed) and separately for each clade. The significance ($P < 0.05$) of the fixation indices was calculated based on 1000 permutations.

Results

Fucus phylogeny

Our nuclear phylogeny agreed well with the morphological delimitation of *Fucus ceranoides*, whereas the organellar phylogenies did not (Fig. 1). The mtIGS and cpORF501 trees revealed two well-supported clades, where southern (Iberian) *F. ceranoides* emerged as sister to a complex polytomy of *F. vesiculosus*, *F. spiralis* and northern *F. ceranoides*. Northern *F. ceranoides* individuals shared their mtDNA and cpDNA sequences with *F. vesiculosus* individuals from Norway and Iceland, ultimately generating disparate levels of intraspecific genetic divergence within *F. ceranoides* and anomalous paraphyletic relationships with respect to the other *Fucus spp.* The position of these northern *F. ceranoides* samples in the nuclear/morphological and organelle-based phylogenies was incongruent. Otherwise, trees simultaneously supported the monophyly of *F. ceranoides* and its clear demarcation from the *F. vesiculosus/F. spiralis* complex.

Fucus ceranoides mtDNA phylogeography

A total of 33 mtIGS haplotypes were identified in 497 sampled individuals of *F. ceranoides*. As expected from the organellar phylogenies, the parsimony analysis revealed two independent networks (clades) displaying nearly disjunct geographic distributions (Table 1 and Fig. 2). The native clade ranged from Portugal to the English Channel and was replaced further North by the introgressed clade. The geographic distribution of both clades overlapped in the Brittany/English Channel area. Both the native and the introgressed clades displayed strong geographical structuring. The native clade was composed of three well-defined phylogeographical lineages. Lineage A and B were restricted to North-western Iberia, and lineage C was distributed from the Bay of Biscay to the English Channel. The introgressed clade comprised a single lineage dominated by two closely related haplotypes, I1 and I9 (63% and 24% of sequences, respectively). I1 (shared with *F. vesiculosus*) and its seven related haplotypes were found from the English Channel to Norway, whereas I9 and its four related haplotypes were confined to northern Wales, Scotland and northern Norway. Only four haplotypes (A1, C1, I1 and I9) were shared among at least two populations, and these were widespread (within phylogroup range) and occupied an interior position in the networks (Fig. 2). All derived haplotypes, diverging by a single (rarely two) mutational steps, were population-specific and thus less abundant. The highest nucleotide diversity of the native clade was present in NW Iberia, although the highest overall

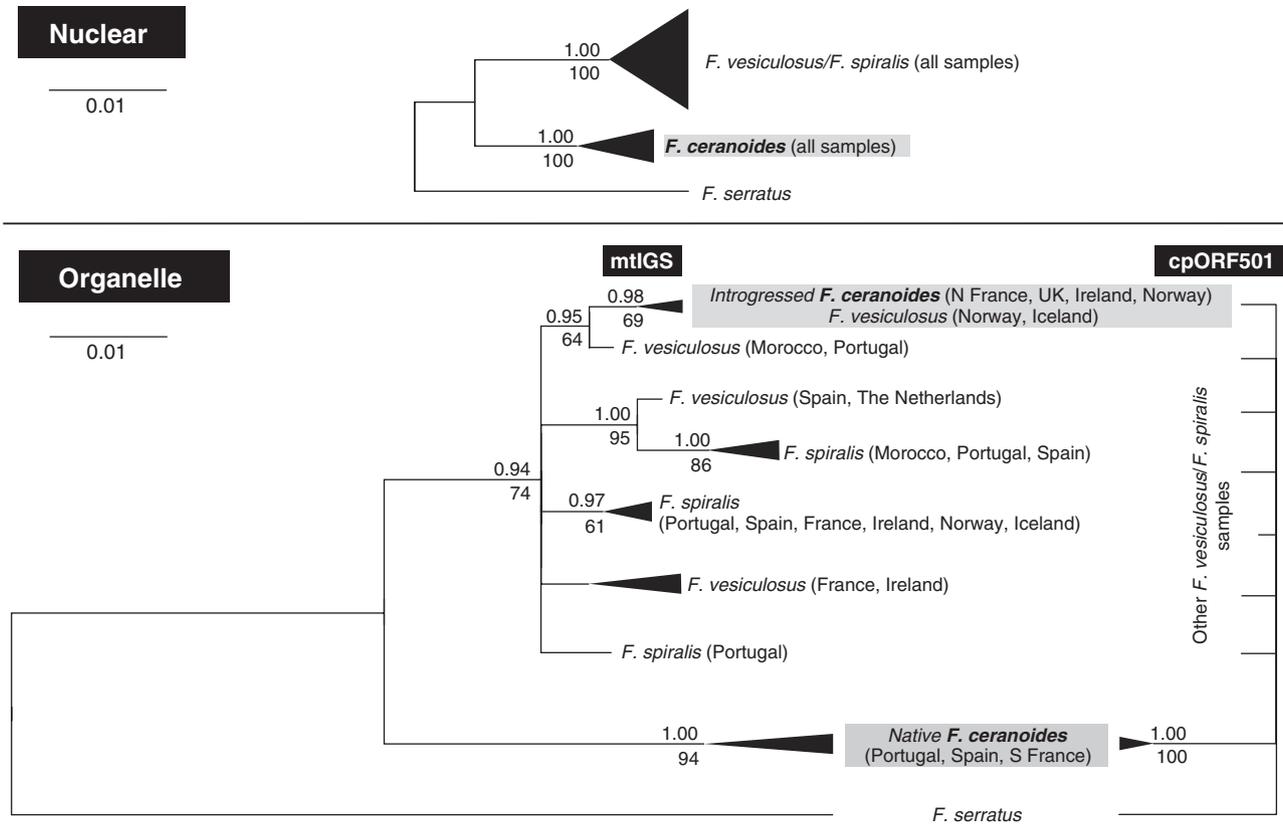


Fig. 1 Bayesian 50% majority-rule consensus trees based on the nPDI (Nuclear) and mtIGS & cpORF501 (Organelle) sequence data. Numbers above and below the branches are Bayesian posterior probabilities (>0.90) and maximum likelihood bootstrap support values (>60), respectively. All trees are built with exactly the same individuals from the three species. For a better visualization, some branches were collapsed (tip triangles). The length (horizontal) of the triangle represents the distance from the branches' common node to the tip of the longest branch, and its height (vertical) is proportional to the number of taxa collapsed. The paraphyletic arrangement of *Fucus ceranoides* in the organelle trees is highlighted in grey.

diversity was located in the Brittany/English Channel area, where the well-differentiated native and introgressed clades co-occurred. The results of the AMOVAS (Table 2) showed that, globally, the native/introgressed groups accounted for about 91% of the molecular variance, whereas only 1.29% of the variation was attributable to differences within populations ($\Phi_{ST} = 0.987$). Within the native and introgressed clades, 89.33% and 69.89% of the molecular variance was accounted for by the molecular differences among respective populations. In all analyses and at all levels, the fixations indices were high to very high ($\Phi > 0.65$).

Discussion

Phylogenetic scope of introgression

Factors that frequently cause discrepancies between gene/species trees and among gene trees include imperfect taxonomy, incomplete lineage sorting of ancestral

polymorphisms and introgression (Takahashi *et al.* 2001; Funk & Omland 2003; Rubinoff & Holland 2005). Here, the divergence levels at all surveyed loci together with the regional sharing of sequences at independently evolving, maternally inherited mtDNA and cpDNA markers (Coyer *et al.* 2002b) clearly indicated the regional introgression of *F. vesiculosus* organelle genomes in the otherwise well-differentiated *F. ceranoides* gene pool.

Although organelle capture is not particularly uncommon, the phylogenetic scope of introgression in *F. ceranoides* is intriguing. First, despite its distinctive estuarine habitat, *F. ceranoides* not uncommonly overlaps, even if very marginally, with *F. vesiculosus* and *F. spiralis* throughout its entire distribution (personal observation). However, divergence levels in all surveyed loci disclose a rather old split between ancestral *F. ceranoides* and *F. vesiculosus/F. spiralis* clades, and are consistent with a general history of reproductive isolation and independent evolution. Second, despite

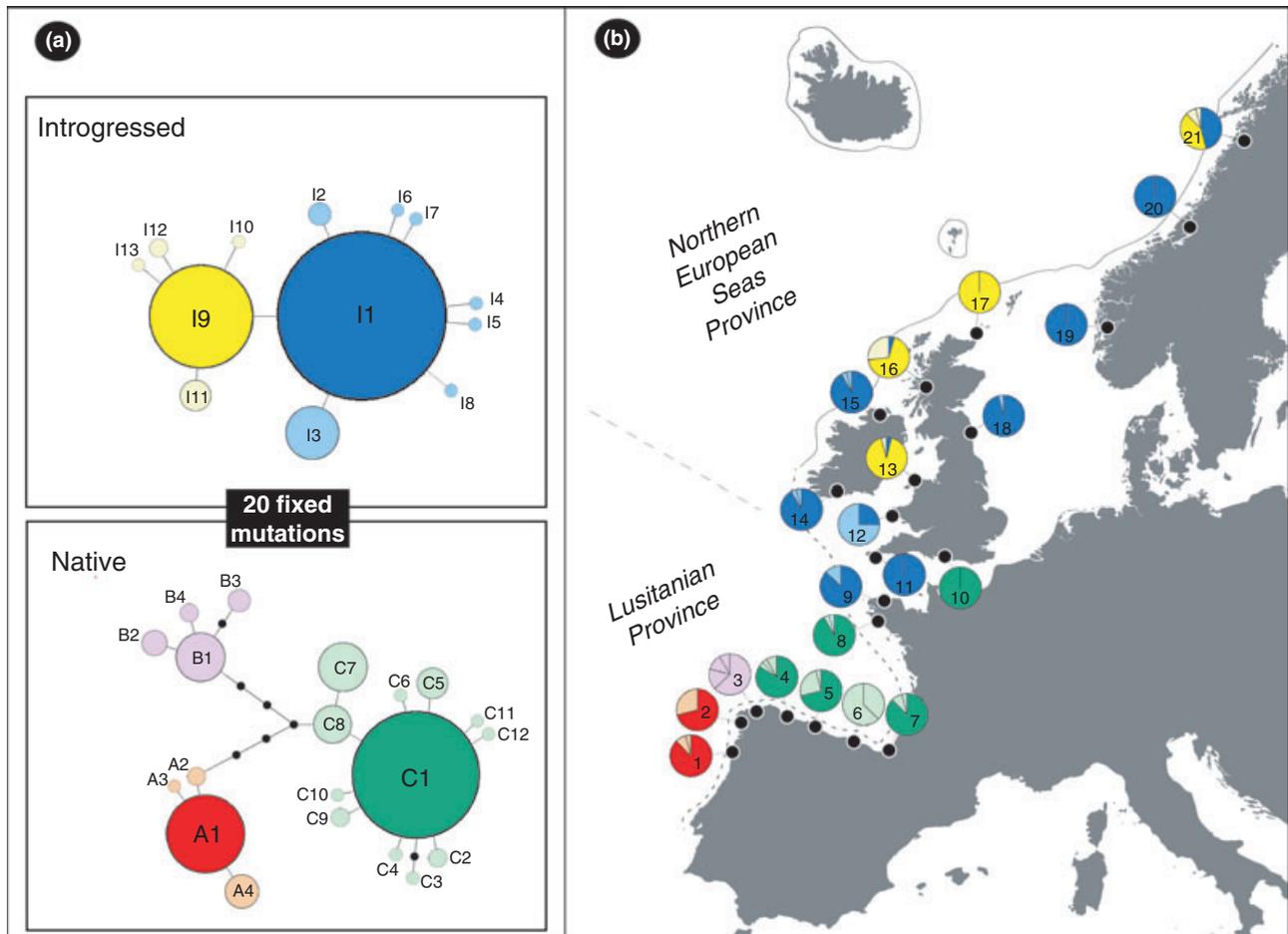


Fig. 2 *Fucus ceranoides* mtIGS haplotypes genealogy and distribution. (a) MtIGS haplotype parsimony networks. Sampled haplotypes are represented by circles sized to their frequency. Black dots represent inferred, unsampled haplotypes. Lineages are labelled by colour and letter [A, B, C and I (two colours)]. Shared and private haplotypes are depicted in bright and pale colour intensity, respectively. (b) Distribution of native and introgressed mtIGS clades. Pie charts depict haplotype frequencies at each site (see Table 1 for location and haplotype ID's). Haplotypes are coloured as in A). Contour line depicts the putative ice-free (dashed) and glaciated (continuous) LGM shoreline (redrawn from Brochmann *et al.* 2003; Menot *et al.* 2006).

Table 2 Results of the hierarchical analyses of molecular variance (AMOVA)

Analysis	N	Level	d.f.	% of total variance	Fixation indices
Native/Introgressed (2 groups)	497	Among groups	1	91.12	$\Phi_{CT} = 0.911^*$
		Among populations within groups	19	7.59	$\Phi_{SC} = 0.854^*$
		Within populations	476	1.29	$\Phi_{ST} = 0.987^*$
Native clade (no groups)	214	Among populations	8	89.33	$\Phi_{ST} = 0.893^*$
		Within populations	205	10.67	
Introgressed clade (no groups)	283	Among populations	11	69.89	$\Phi_{ST} = 0.699^*$
		Within populations	271	30.11	

Populations of *Fucus ceranoides* were grouped according to major mtIGS phylogroups. *P* values are based on 1000 random permutations.

* $P < 0.001$.

the extensive penetration of a single alien *F. vesiculosus* cytoplasm throughout northern Europe, organelle capture is inferred to have occurred rather recently,

because mitochondrial and chloroplast sequences are basically identical between the two species, i.e. they display very low (mtIGS) or null (cpORF501) post-

introgression mutation, and still retain some degree of geographical association (haplotypes are shared throughout the Northern European Seas). Finally, a history of widespread and recurrent hybridization, especially given the extent (but inferred recentness) of organellar introgression, would be expected to leave some trace in the nuclear genome (Martinsen *et al.* 2001; Scotti-Saintagne *et al.* 2004; Yatabe *et al.* 2007). Despite the still limited genomic and geographical coverage, the available molecular data [this study and Billard *et al.* (2005) using nuclear microsatellites] reveal a remarkable integrity of the *F. ceranoides* nuclear background across introgressed and nonintrogressed populations and provide no detectable evidence of on-going gene-flow with sympatric *F. vesiculosus* (i.e. disjunct distribution of alleles between the species at two loci). This inferred rarity of hybrid bridges makes it very unlikely that recurrent (local) organellar gene-flow should be the primary cause for the prevalence of an alien cytoplasm throughout the northern part of *F. ceranoides* range.

Post-glacial range expansion and spread of the introgressed organellar genomes

The highest diversity and endemism of native lineages of *F. ceranoides* is located in NW Iberia, a putative Pleistocene climatic refugium currently at the rear edge of its distribution. East and northwards vast disjunct areas are dominated by lineages C and I, more specifically by their interior (putative ancestral) haplotypes C1 and I1. These geographically structured lineages occupy the latitudes that were most severely affected by the advance of the polar front during the last glacial maximum (LGM, ~20,000 BP) and, as expected, display a shallow star-like topology consistent with a recent demographic expansion. The cold and arid LGM climate caused major latitudinal shifts in terrestrial biomes (Hewitt 2000; Brochmann *et al.* 2003; Lomolino *et al.* 2005) and LGM sea surface temperature reconstructions place the 10 °C summer isotherm, the current northern range limit of *F. ceranoides* (Lein 1984), from Brittany southwards (Meland *et al.* 2005 and references therein). Although the characteristics of the mtLGS marker (introgression, low polymorphism and extreme structuration) make it unsuitable for formally testing such a range expansion into northern latitudes (e.g. using mismatch or coalescent-based approaches), the prevailing paleoclimatic conditions dictate that the colonization of the northern European estuaries could only have taken place at the onset of the last deglaciation, when the ice-sheets retreated, the isotherms shifted northwards and the present hydrological system was resumed (Menot *et al.* 2006). Such post-gla-

cial, northwards range expansions are paradigmatic among the temperate terrestrial biota (Hewitt 2000) and were already reported for several other marine algae (Provan *et al.* 2005; Hoarau *et al.* 2007).

Introgressed and nonintrogressed populations have an essentially disjunct distribution, overlapping in a narrow transition zone around the English Channel that spans only 3° of the c. 30° latitudinal range of *F. ceranoides*. Noticeably, the range of each clade matches the Lusitanian and the Northern European Seas marine provinces (Spalding *et al.* 2007), raising the possibility that positive selection was involved in the replacement of the organellar genomes along this biogeographic transition zone. This selective sweep hypothesis would require that at least one locus in either mtDNA or cpDNA of *F. vesiculosus* was advantageous in both *F. vesiculosus* and *F. ceranoides* nuclear backgrounds, possibly by contributing to adaptation to the colder environments of Northern Europe (Ruiz-Pesini *et al.* 2004; Melo-Ferreira *et al.* 2005; Ballard & Melvin 2010). Neither molecular nor direct physiological evidence is presently available to support or reject such adaptive advantage, but the phylogeographic break in the English Channel area is also compatible with the stochastic surfing of the introgressed organellar genomes during *F. ceranoides* post-glacial range expansion (Excoffier & Ray 2008; Hofer *et al.* 2009). Empirical evidence and model simulations have shown that strong genetic drift at expanding margins can promote purely stochastic and neutral sweeps in genetic variation, and that surfing of particular alleles can result in the geographic segregation of distinct alleles within a species range (Klopfstein *et al.* 2006; Hallatschek *et al.* 2007; Excoffier & Ray 2008), even if they confer no selective advantage. Simulations that extend these models to expanding taxa that interbreed with locally established species (Currat *et al.* 2008) further demonstrate that alleles introgressing close to the leading edge of an expansion can surf just as new intraspecific mutations do (Edmonds *et al.* 2004; Klopfstein *et al.* 2006). Thus, the extensive dissemination of introgressed organellar genomes during *F. ceranoides* range expansion should not a priori be interpreted as a sign of selection, but rather as the null expectation for a neutral gene (Currat *et al.* 2008).

Under this scenario, locally introgressed organellar genomes increased their frequency in *F. ceranoides* by chance or adaptive selection at the leading edge of its expansion, presumably around the English Channel, and this position then favoured their unique spread throughout northern Europe (i.e. genetic surfing). This would be a relatively simple process in this system, for several reasons. *F. ceranoides* has a linear distribution along the coast, resulting in narrow wave fronts. Genetic surfing is also facilitated in small and fast

growing populations and when intra-specific gene-flow is limited between neighbouring populations (Klopfstein *et al.* 2006; Petit & Excoffier 2009). These characteristics apply well to this alga, which lacks dispersive planktonic stages and occurs in spatially discrete estuaries frequently isolated by dozens to hundreds of kilometres of coastline. Gene flow between estuaries is clearly limited as revealed by the absence of mixed lineages even at very short spatial scales (extremely high Φ_{SC} values), suggesting that founder effects and mutation/drift are more important than gene-flow in determining the genetic make-up of fully established populations. Ultimately, the observed phylogeographic break would only require a single replacement of the native organelles (containing haplotype C1) by those of *F. vesiculosus* (containing haplotype I1) at the expansion front, followed by northward spread during the post-glacial range expansion. A similar process occurred around northern Wales where introgressed haplotype I1 was replaced by its derived I9 haplotype (in this case originated via mutation, not introgression), which eventually colonized the contiguous western coast of Scotland.

The surfing hypothesis also explains the restricted genomic and taxonomic scope of introgression. The propagation of native or alien allelic variants across a geographic range by surfing phenomena is more likely to occur when the range of a species is expanding than in stationary populations. The 'asymmetric' flow of organelles, exclusively in the direction of *F. ceranoides* (Fig. 1; Coyer *et al.* 2006) is likely to reflect the different demographic status of *F. ceranoides* and *F. vesiculosus* dating from the time of introgression. *F. vesiculosus* survived the LGM around the Brittany/English Channel paleo-coastline (Coyer *et al.* unpublished data) and was thus fully established when *F. ceranoides* arrived in this area. The dissemination of any *F. ceranoides* allele from introgressants would be restrained by competition with conspecifics rather than magnified by population growth, increasing its probabilities of remaining either rare and geographically restricted, or lost by drift. In addition, many animal (mtDNA) and plant (cpDNA) studies reveal that organellar genomes frequently penetrate foreign gene-pools more extensively than nuclear genes (Dumolin-Lapegue *et al.* 1999; Shaw 2002; Roca *et al.* 2005; Berthier *et al.* 2006; Good *et al.* 2008). The effective population size (N_e) of these genomes is generally $\frac{1}{4}$ that of nuclear autosomal loci, and thus lineage sorting is expected to progress more rapidly (Palumbi *et al.* 2001). For the same reason, unless gene-flow is high or female-biased, maternally transmitted organellar genomes are expected to be more prone to surf (and to invasion) than nuclear genes (Petit & Excoffier 2009), which should, at least partially, account for the integrity of *F. ceranoides* nuclear background despite organellar

replacement. Naturally, as *F. ceranoides* and its marine congeners occupy distinct habitats, biased backcrossing and selection must also have had a role in preserving the parental nuclear genotypes. However, even strong purifying selection against nonadaptive nuclear alleles from introgressants would not counteract the exchange and spread of unlinked, nonconflicting, clonally transmitted organellar genomes.

Conclusions

The genetic surfing of alien alleles during spatial expansions provides a general mechanism for extensive cytoplasmic introgression, even without invoking a better performance for nonco-evolving organellar/nuclear genome combinations or recurrent hybridization. In our view, it represents the most parsimonious explanation for the replacement of native *F. ceranoides* organellar genomes by an alien cytoplasm throughout northern Europe. If our case study supports a role for such nonequilibrium demographic processes in providing a highway for the spread of introgressed material, it remains to be seen how frequent such phenomena are in the marine realm. Marine species and communities readily respond to climate change (Breeman 1990; Beaugrand *et al.* 2002; Perry *et al.* 2005), and many certainly experienced spatial expansions associated with past climatic-driven range shifts. Because hybridization in the sea is not particularly uncommon (Coyer *et al.* 2002a; Addison & Hart 2005; Willis *et al.* 2006; Kuriwa *et al.* 2007), it seems rather surprising that almost all described instances of extensive introgression come from terrestrial and freshwater taxa. As pointed earlier, surfing phenomena are facilitated in species (or markers) that experience low levels of intraspecific gene-flow. For many, if not most marine species, the existence of planktonic propagule stages and the typical disconnection between propagule production and settlement is likely to prevent the emergence of sharp phylogeographic patterns. Therefore, we predict that surfing phenomena, and particularly those leading to extensive introgression, are less common in the sea and most likely to be found in taxa sharing some of the life history characteristics of *F. ceranoides* dependence on a fragmented habitat, lack of planktonic stages and essentially self-recruiting populations.

More generally, our study shows that an adequate genomic and taxonomic sampling is of particular importance when introgression is pervasive. A recent mtDNA phylogeny of *Fucus* (Coyer *et al.* 2006) was unable to recognize *F. ceranoides* as an early off-shoot from the lineage leading to *F. vesiculosus* and *F. spiralis*, because it only included individuals from northern

Europe, fixed for the introgressed *F. vesiculosus* haplotypes. Similarly, we would not be able to recognize the reticulate history of *F. ceranoides* and fully interpret the source of its intra-specific genetic variation and phylogeographic structuring without sampling additional species and a nuclear gene, i.e. without the incorporation of a broader phylogenetic perspective.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Geographic origin of *Fucus* spp. samples ($N = 35$) sequenced for the nPDI, mtIGS and cpORF501 loci

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